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(54) Title: IMMUNOGLOBULINS

(57) Abstract: The present invention relates to antibodies to NOGO, pharmaceutical formulations containing them and to the use
of such antibodies in the treatment and/or prophylaxis of neurological diseases/disorders.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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Immunoglobulins**Field of the Invention**

The present invention relates to immunoglobulins, particularly antibodies that bind to NOGO and neutralise the activity thereof, polynucleotides encoding such antibodies, pharmaceutical formulations containing said antibodies and to the use of such antibodies in the treatment and/or prophylaxis of neurological diseases. Other aspects, objects and advantages of the present invention will become apparent from the description below.

Background of the Invention

Stroke is a major cause of death and disability in the Western World. There is no approved therapy for the treatment of stroke other than tissue plasminogen (t-PA) which has to be administered within 3 hours of onset following a computer tomography (CT) scan to exclude haemorrhage. To date most therapeutic agents directed towards the treatment of acute stroke (i.e. neuroprotection), have predominantly involved targeting glutamate receptors and their down stream signalling pathways known to be involved in acute cell death. However to date these strategies have proved unsuccessful in clinical trials and are often associated with dose-limiting side effects (Hill & Hachinski, The Lancet, 352 : (suppl III) 10-14 (1998)). Therefore there is a need for novel approaches directed towards the amelioration of cell death following the cessation of blood flow. Neuroprotection is the ability of a treatment to prevent or ameliorate neuronal cell loss in response to an insult or disease process. This may be achieved by targeting the neurons directly or indirectly by preventing glial (including oligodendrocyte) cell loss.

Following the onset of stroke, some degree of spontaneous functional recovery is observed in many patients, suggesting that the brain has the (albeit limited) ability to repair and/or remodel following injury. Agents that have the potential to enhance this recovery may therefore allow intervention to be made much later (potentially days) following the onset of cerebral ischaemia. Agents which are able to offer both acute neuroprotection and enhance functional

5 recovery may provide significant advantages over current potential neuroprotective strategies.

Alzheimer's disease (AD) is characterised by the presence of two diagnostic features of pathology. These are amyloid plaques and neurofibrillary
10 tangles composed of aggregated beta-amyloid peptide (A β 40 and A β 42) and hyperphosphorylated tau respectively (Dawbarn & Allen 2001 Neurobiology of Alzheimer's Disease OUP).

A comprehensive study has shown a strong link in patients between beta-amyloid accumulation and cognitive decline (Naslund et al, JAMA, March 22/29,
15 2000, Vol.283, No;12, page 1571-1577). This is consistent with genetic and epidemiological studies that suggest that some mutations in APP and presenilin genes can predispose to early onset AD, which mutations also enhance the levels of A β 40 and A β 42 peptide, including the ratio thereof.

Cleavage of the type I transmembrane amyloid precursor protein (APP) by
20 two distinct proteases designated beta- and gamma-secretase is necessary for the formation of beta-amyloid peptide. The molecular identity of beta-secretase as the aspartyl-protease Asp2/BACE1 has been confirmed (Hussain et al Mol.Cell.NeuroSci. 16, 609-619 (2000); Vassar et al, Science (1999), Oct.22; 286 (5440):735-741). The nature of gamma-secretase remains the source of some
25 debate and is likely to consist of a high molecular weight complex consisting of at least the following proteins: presenilins, Aph1, Pen2 and nicastrin (reviewed in Medina & Dotti Cell Signalling 2003 15(9):829-41).

The processing of APP within the CNS is likely to occur within a number of cell-types including neurons, oligodendrocytes, astrocytes and microglia. While
30 the overall rate of APP processing in these cells will be influenced by the relative level of expression of APP, BACE1/Asp2, presenilin-1 and -2, Aph1, Pen2 and nicastrin.

Furthermore, additional factors regulating the subcellular location of APP can also influence its processing as shown by the finding that mutation of the
35 YENP motif in the APP cytoplasmic domain which blocks its endocytosis reduces beta-amyloid production (Perez et al 1999 J Biol Chem 274 (27) 18851-6). Retention of the APP-beta-CTF in the ER by the addition of the KKQN retention

5 motif is sufficient to reduce amyloid production in transfected cells (Maltese et al 2001 J Biol Chem 276 (23) 20267-20279). Conversely, elevation of endocytosis, by overexpression of Rab5 is sufficient to elevate amyloid secretion from transfected cells (Grbovic et al 2003 J Biol Chem 278 (33) 31261-31268).

10 Consistent with these findings further studies have shown that reduction of cellular cholesterol levels (a well known risk factor for AD) reduced beta-amyloid formation. This change was dependent on altered endocytosis as demonstrated by the use of the dominant negative dynamin mutants (K44A) and overexpression of the Rab5 GTPase activating protein RN-Tre (Ehehalt et al
15 2003 J Cell Biol 160 (1) 113-123).

Cholesterol rich microdomains or rafts are also an important cellular site of beta-amyloid production and APP, BACE1 and components of the gamma-secretase complex have all been shown to transiently reside within rafts. Antibody cross-
20 linking of APP and BACE1 towards cholesterol rich rafts was able to elevate beta-amyloid production (Ehehalt et al 2003 J Cell Biol 160 (1) 113-123). Expression of GPI-anchored BACE1, which is exclusively targeted to lipid rafts, is similarly able to elevate APP cleavage and beta-amyloid production (Cordy et al 2003 PNAS 100(20) 11735-11740).

25

The mechanisms underlying functional recovery are currently unknown. The sprouting of injured or non-injured axons has been proposed as one possible mechanism. However, although in vivo studies have shown that treatment of spinal cord injury or stroke with neurotrophic factors results in enhanced
30 functional recovery and a degree of axonal sprouting, these do not prove a direct link between the degree of axonal sprouting and extent of functional recovery (Jakeman, et al. 1998, Exp. Neurol. 154: 170-184, Kawamata et al. 1997, Proc Natl Acad. Sci. USA., 94:8179-8184, Ribotta, et al. 2000, J Neurosci. 20: 5144-5152). Furthermore, axonal sprouting requires a viable neuron. In diseases such
35 as stroke which is associated with extensive cell death, enhancement of functional recovery offered by a given agent post stroke may therefore be through mechanisms other than axonal sprouting such as differentiation of

5 endogenous stem cells, activation of redundant pathways, changes in receptor distribution or excitability of neurons or glia (Fawcett & Asher, 1999, Brain Res. Bulletin, 49: 377-391, Horner & Gage, 2000, Nature 407 963-970).

The limited ability of the central nervous system (CNS) to repair following injury is thought in part to be due to molecules within the CNS environment that
 10 have an inhibitory effect on axonal sprouting (neurite outgrowth). CNS myelin is thought to contain inhibitory molecules (Schwab ME and Caroni P (1988) J. Neurosci. 8, 2381-2193). Two myelin proteins, myelin-associated glycoprotein (MAG) and NOGO have been cloned and identified as putative inhibitors of neurite outgrowth (Sato S. et al (1989) Biochem. Biophys. Res. Comm. 163, 1473-
 15 1480; McKerracher L et al (1994) Neuron 13, 805-811; Mukhopadhyay G et al (1994) Neuron 13, 757-767; Torigoe K and Lundborg G (1997) Exp. Neurology 150, 254-262; Schafer et al (1996) Neuron 16, 1107-1113; WO9522344; WO9701352; Prinjha R et al (2000) Nature 403, 383-384; Chen MS et al (2000) Nature 403, 434-439; GrandPre T et al (2000) Nature 403, 439-444;
 20 US005250414A; WO200005364A1; WO0031235).

Three forms of human NOGO have been identified: NOGO-A having 1192 amino acid residues (GenBank accession no. AJ251383); NOGO-B, a splice variant which lacks residues 186 to 1004 in the putative extracellular domain (GenBank accession no. AJ251384) and a shorter splice variant, NOGO-C,
 25 which also lacks residues 186 to 1004 and also has smaller, alternative amino terminal domain (GenBank accession no. AJ251385) (Prinjha et al (2000) supra).

Inhibition of the CNS inhibitory proteins such as NOGO may provide a therapeutic means to ameliorate neuronal damage and promote neuronal repair and growth thereby potentially assisting recovery from neuronal injury such as
 30 that sustained in stroke. Examples of such NOGO inhibitors may include small molecules, peptides and antibodies.

Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable
 35 domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain.

- 5 The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. The variable domains on the light and heavy chains have the same general structure and each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs) often referred to as hypervariable regions. The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site. CDRs and framework regions of antibodies may be determined by reference to Kabat et al ("Sequences of proteins of immunological interest" US Dept. of Health and Human Services, US Government Printing Office, 1987).

It has been found that anti-MAG monoclonal antibodies, described (Poltorak et al (1987) Journal of Cell Biology 105,1893-1899, DeBellard et al (1996) Mol. Cell. Neurosci. 7, 89-101; Tang et al (1997) Mol. Cell. Neurosci. 9, 333-346; Torigoe K and Lundborg G (1997) Exp. Neurology 150, 254-262) and commercially available (MAB1567 (Chemicon)) when administered either directly into the brain or intravenously following focal cerebral ischaemia in the rat (a model of stroke), provides neuroprotection and enhances functional recovery (PCT/EP03/08749).

Therefore anti-MAG antibodies provide potential therapeutic agents for both acute neuroprotection as well as the promotion of functional recovery following stroke. This antibody is a murine antibody. Although murine antibodies are often used as diagnostic agents their utility as a therapeutic has been proven in only a few cases. Their limited application is in part due to the repeated administration of a murine monoclonal antibody to a human usually elicits human immune responses against these molecules. To overcome these intrinsic undesirable properties of murine antibodies, "altered" antibodies designed to incorporate regions of human antibodies have been developed and

5 are well established in the art. For example, a humanised antibody contains complementarity determining regions ("CDR's") of non human origin and the majority of the rest of the structure is derived from a human antibody.

It has also been reported that a murine monoclonal antibody, IN-1, that was raised against NI-220/250, a myelin protein which is a potent inhibitor of neurite growth (and subsequently shown to be fragment of NOGO-A), promotes axonal regeneration (Caroni, P and Schwab, ME (1988) Neuron 1 85-96; Schnell, L and Schwab, ME (1990) Nature 343 269-272; Bregman, BS et al (1995) Nature 378 498-501 and Thallmair, M et al (1998) Nature Neuroscience 1 124-131). It has also been reported that NOGO-A is the antigen for IN-1 (Chen et al (2000) Nature 403 434-439). Administration of IN-1 Fab fragment or humanised IN-1 to rats that have undergone spinal cord transection, enhanced recovery (Fiedler, M et al (2002) Protein Eng 15 931-941; Brosamle, C et al (2000) J. Neuroscience 20 8061-8068). However to date there is no evidence in the literature to suggest that IN-1, or its humanised form, can bind and inhibit human NOGO-A, a necessary requirement for a monoclonal antibody to be useful in the therapeutic treatment of NOGO-mediated diseases and disorders such as stroke and neurodegenerative diseases in humans.

Therefore it remains a highly desirable goal to isolate and develop a therapeutically useful monoclonal antibody that binds and inhibits the activity of human NOGO. The process of neurodegeneration underlies many neurological diseases/disorders including, but not limited to, acute diseases such as stroke (ischemic or haemorrhagic), traumatic brain injury and spinal cord injury as well as chronic diseases including Alzheimer's disease, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Creutzfeldt-Jakob disease (CJD), Schizophrenia, amyotrophic lateral sclerosis (ALS), multiple sclerosis, Huntington's disease, multiple sclerosis and inclusion body myositis.

Consequently an anti-NOGO monoclonal antibody may be useful in the treatment of these diseases/disorders. Such antibodies for the treatment of the above mentioned disease/disorders are provided by the present invention and described in detail below.

- 5 All publications, both journal and patent, disclosed in this present specification are expressly and entirely incorporated herein by reference.

Brief Summary of the Invention

- 10 The invention provides an antibody or functional fragment thereof which binds to and neutralises the activity of NOGO, preferably human NOGO, more preferably human NOGO-A (sometimes referred to herein as "anti-NOGO antibody"). Such antibody may, for example, comprise one or more CDR's as shown in the tables 1 to 6 which show the CDRs of three such independently
15 isolated antibodies: 2A10/3, 2C4/1 and 15C3/3. The CDR's are identified as described by Kabat (Kabat et al. (1991) "Sequences of proteins of immunological interest"; Fifth Edition; US Department of Health and Human Services; NIH publication No 91-3242). CDRs preferably are as defined by Kabat but following the principles of protein structure and folding as defined by Chothia and Lesk,
20 (Chothia et al., (1989) "Conformations of immunoglobulin hypervariable regions"; Nature 342, p877-883) it will be appreciated that additional residues may also be considered to be part of the antigen binding region and are thus encompassed by the present invention.

- 25 Table 1: Antibody 2A10/3 ("2A10") light chain CDRs

CDR	According to Kabat
L1	RSSKSLLYKDGKTYLN (SEQ ID NO:1)
L2	LMSTRAS (SEQ ID NO:2)
L3	QQLVEYPLT (SEQ ID NO:3)

Table 2: Antibody 2A10/3 heavy chain CDRs

30

CDR	According to Kabat
H1	SYWMH (SEQ ID NO:4)

H2	NINPSNGGTNYNEKFKS (SEQ ID NO:5)
H3	GQGY (SEQ ID NO:6)

5

Table 3: Antibody 2C4/1 ("2C4") light chain CDRs

CDR	According to Kabat
L1	RSSQSLVHSNGNTYLH (SEQ ID NO:7)
L2	KVSNRFS (SEQ ID NO:8)
L3	SQSTHVPLT (SEQ ID NO:9)

10 Table 4: Antibody 2C4/1 heavy chain CDRs

CDR	According to Kabat
H1	FSCYAMS (SEQ ID NO:10)
H2	SISDGGSYTYYPDNVKG (SEQ ID NO:11)
H3	ELLFDY (SEQ ID NO:12)

Table 5: Antibody 15C3/3 ("15C3") light chain CDRs

CDR	According to Kabat
L1	RSSKSLVHSNGNTYLY (SEQ ID NO:13)
L2	RMSNLAS (SEQ ID NO:14)
L3	MQHLEYPLT (SEQ ID NO:15)

15

Table 6: Antibody 15C3/3 heavy chain CDRs

CDR	According to Kabat
H1	SYWMN (SEQ ID NO:16)
H2	QIYPGDGDTNYNGKFKG (SEQ ID NO:17)
H3	RFDY (SEQ ID NO:18)

5

In a first aspect, the present invention provides:

- 10 (a) An antibody or functional fragment thereof which binds to and neutralises NOGO, particularly human NOGO, more particularly human NOGO-A activity which antibody or functional fragment thereof comprises a heavy chain variable domain which comprises each of the CDR's of table 2, and a light chain variable domain which comprises each of the CDRs from table 1.
- 15 (b) An antibody or functional fragment thereof which binds to and neutralises NOGO, particularly human NOGO, more particularly human NOGO-A activity which antibody or functional fragment thereof comprises a heavy chain variable domain which comprises each of the CDR's of table 4 and a light chain variable domain which comprises each of the CDRs of table 3.
- 20 (c) An antibody or functional fragment thereof which which antibody or functional fragment thereof comprises a heavy chain variable region which comprises each of the CDR's selected of table 6 and a light chain variable domain which comprises each of the CDRs of table 5.
- 25

We further provide an anti-NOGO antibody or functional fragment thereof which comprises:

- 30 a) a heavy chain variable domain (V_H) which comprises in sequence CDRH1, CDRH2 and CDRH3 from table 2,
and /or
b) a light chain variable domain (V_L) which comprises in sequence CDRL1, CDRL2 and CDRL3 from table 1;

an anti-NOGO antibody or functional fragment thereof which comprises:

- 35 a) a heavy chain variable domain (V_H) which comprises in sequence CDRH1, CDRH2 and CDRH3 from table 4,
and /or

- 5 b) a light chain variable domain (V_L) which comprises in sequence
CDRL1, CDRL2 and CDRL3 from table 3; or
an anti-NOGO antibody or functional fragment thereof which comprises:
- a) a heavy chain variable domain (V_H) which comprises in sequence
CDRH1, CDRH2 and CDRH3 from table 6,
- 10 and /or
- c) a light chain variable domain (V_L) which comprises in sequence
CDRL1, CDRL2 and CDRL3 from table 5.

15 The antibody maybe chimeric, fully human, or humanised.

In another aspect, the present invention also relates to an anti-NOGO antibody which binds to the same (or overlapping) epitope on the NOGO polypeptide as an antibody having the heavy and light chain variable regions described above. Preferably the epitope is comprised within the region 586 to 785 (NOGO-A amino acid numbering, Genbank accession number AJ251383), more preferably the epitope is comprised within the region 586 to 685 or 686 to 785. Competitive inhibition assays are used for mapping of the epitopes on an antigen. Thus there is also provided an anti-NOGO antibody which binds to the human NOGO-A between amino acids 586 to 685 or 686 to 785 and neutralises the activity of NOGO-A. Such an antibody may be produced according to the methods set forth below. In a further aspect, there is also provided an anti-NOGO antibody which competitively inhibits the binding of the antibody having the CDRs described supra to NOGO, preferably human NOGO, most preferably human NOGO-A.

More specifically there is provided an antibody, which may be fully human, humanised or chimeric which binds to and neutralises the activity of NOGO, particularly human NOGO, more particularly human NOGO-A which competitively inhibits, at equimolar concentration, the binding to human NOGO-A of an antibody having a heavy chain variable region comprising each of the CDR's of Table 2 and a light chain variable region comprising each of the CDR's of Table 1.

5

In another embodiment, there is provided an antibody, which may be fully human, humanised or chimeric which binds to and neutralises the activity of NOGO, particularly human NOGO, more particularly human NOGO-A which competitively inhibits the binding to human NOGO-A of an antibody having a heavy chain variable region comprising each of the CDR's of Table 4 and a light chain variable region comprising each of the CDR's of Table 3.

In another embodiment there is provided an antibody, which may be fully human, humanised or chimeric which binds to and neutralises the activity of NOGO, particularly human NOGO, more particularly human NOGO-A which competitively inhibits the binding to human NOGO-A of an antibody having a heavy chain variable region comprising each of the CDR's of Table 6 and a light chain variable region comprising each of the CDR's of Table 5.

20

In typical embodiments, the competing antibody is of the IgG class, more typically IgG1 or IgG4.

Chimeric Antibodies

Also provided is a chimeric antibody which binds to and neutralises NOGO, preferably human NOGO, more preferably human NOGO-A comprising CDRs such as those disclosed in tables 1 to 6. Preferably the chimeric antibody comprises mouse and human sequences (e.g. mouse variable region and human constant region). Moreover there is provided a chimeric antibody comprising a heavy chain variable region comprising each of the CDR's of Table 2 and a light chain variable region comprising each of the CDRs of Table 1.

30

There is also provided a chimeric antibody comprising a heavy chain variable region comprising each of the CDR's of Table 4 and a light chain variable region comprising each of the CDRs of Table 3.

35

- 5 There is also provided a chimeric antibody comprising a heavy chain variable region comprising each of the CDR's of Table 6 and a light chain variable region comprising each of the CDRs of Table 5.

10 In typical embodiments, the competing antibody is of the IgG class, more typically human IgG1 or IgG4, with a κ type human light chain.

Humanised Antibodies

15 Further, the invention also provides a humanised antibody which binds to and neutralises NOGO, preferably human NOGO, more preferably human NOGO-A.

20 More specifically there is provided a humanised antibody comprising a heavy chain variable region comprising each of the CDR's of Table 2 and a light chain variable region comprising each of the CDRs of Table 1.

25 There is also provided a humanised antibody comprising a heavy chain variable region comprising each of the CDR's of Table 4 and a light chain variable region comprising each of the CDRs of Table 3.

There is also provided a humanised antibody comprising a heavy chain variable region comprising each of the CDR's of Table 6 and a light chain variable region comprising each of the CDRs of Table 5.

30 In typical embodiments, the antibodies of the invention, whether they are chimaeric, humanised or fully human are of the IgG class, more typically human IgG1 or IgG4, with a κ type human light chain.

35 A further aspect of the invention provides a pharmaceutical composition comprising an anti-NOGO antibody of the present invention or functional fragment thereof together with a pharmaceutically acceptable diluent or carrier.

5 In a further aspect, the present invention provides a method of treatment or prophylaxis of stroke (particularly ischemic stroke) and other neurological diseases, in particular Alzheimer's disease, in a human which comprises administering to said human in need thereof an effective amount of an anti-NOGO antibody of the invention or functional fragments thereof.

10 In another aspect, the invention provides the use of an anti-NOGO antibody of the invention or a functional fragment thereof in the preparation of a medicament for treatment or prophylaxis of stroke (particularly ischemic stroke) and other neurological diseases, in particular Alzheimer's disease.

15 In a further aspect, the present invention provides a method of inhibiting neurodegeneration and/or promoting functional recovery in a human patient afflicted with, or at risk of developing, a stroke (particularly ischemic stroke) or other neurological disease, in particular Alzheimer's disease, which comprises administering to said human in need thereof an effective amount of an anti-NOGO antibody of the invention or a functional fragment thereof.

20 In a yet further aspect, the invention provides the use of an anti-NOGO antibody of the invention or a functional fragment thereof in the preparation of a medicament for inhibiting neurodegeneration and/or promoting functional recovery in a human patient afflicted with, or at risk of developing, a stroke and other neurological disease, in particular Alzheimer's disease.

25 In a further aspect, we provide an antibody or functional fragment thereof which comprises a heavy chain variable domain which comprises one or more CDR's selected from CDRH1, CDRH2 and CDRH3 of table 1, preferably comprising at least CDRH3, and/or a light chain variable domain which
30 comprises one or more CDRs selected from CDRL1, CDRL2 and CDRL3 from table 4; an antibody or functional fragment thereof which comprises a heavy chain variable domain which comprises one or more CDR's selected from CDRH1, CDRH2 and CDRH3 of table 2, preferably comprising at least CDRH3, and/or a light chain variable domain which comprises one or more CDRs
35 selected from CDRL1, CDRL2 and CDRL3 from table 5; or an antibody or functional fragment thereof which comprises a heavy chain variable domain which comprises one or more CDR's selected from CDRH1, CDRH2 and CDRH3

5 of table 3, preferably comprising at least CDRH3, and/or a light chain variable domain which comprises one or more CDRs selected from CDRL1, CDRL2 and CDRL3 from table 6.

Other aspects and advantages of the present invention are described further in the detailed description and the preferred embodiments thereof.

10

Description of the Figures

Figure 1 shows the inhibitory effect of the GST-NOGO-A56 fusion protein on neurite outgrowth. The Y axis shows the average neurite length/ neurite (NL/N) in arbitrary units.

15

Figure 2 shows the blocking effect by the supernatant of the hybridoma 2A10 on the neurite outgrowth inhibitory activity of NOGO-A56 (GST-Nogo5&6). The Y axis is as for figure 1.

20 Figure 3 shows the blocking effect by the supernatant of the hybridoma 2C4 on the neurite outgrowth inhibitory activity of NOGO-A56 (GST-Nogo5&6). The Y axis is as for figure 1.

25 Figure 4 shows the blocking effect by the supernatant of the hybridoma 15C3 on the neurite outgrowth inhibitory activity of NOGO-A56 (GST-Nogo5&6). The Y axis is as for figure 1.

Figure 5 is the control hybridoma supernatant 12G3 which has no NOGO-A56 blocking activity. The Y axis is as for figure 1.

30

Figure 6 shows the NOGO-A56-blocking effect of purified 2A10 at 4 concentrations. The Y axis is as for figure 1.

35 Figure 7 shows that recombinant IN-1 monoclonal antibody does not show any blocking activity towards NOGO-A56 (GST-NOGO5&6). Y axis is as for figure 1.

For figures 1 to 7 the negative control is the GST protein alone.

5

Figure 8 shows the binding of 2A10, 2C4 and 15C3 monoclonal antibodies to human NOGO-A56. The Y-axis shows the measured OD at 450nm, a quantitative measure of antibody captured in the wells. The X-axis shows the concentration of antibody used (ng/ml) per well at each data point.

10

Figure 9 shows the lesion volume as a percentage of total brain volume at various concentrations following the study of example 10.

Figure 10 shows neuroscore data of example 10 represented as means \pm SEM.

15

Figures 11A, 11B, 11C, 11D. Cylinder data of example 10 represented as mean \pm SEM for A) both paws, B) left paw, C) right paw and D) right paw split into rats that received 3 doses of 15 μ g of anti-NOGO antibody, and those which received 4 doses of anti-NOGO antibody.

20

Figure 12 shows forelimb foot slips of example 10 represented as mean \pm 95% confidence intervals.

Figure 13 shows hindlimb footslips of example 10 represented as mean \pm 95 %

25

confidence intervals.

Figure 14 A) shows body weights represented as means \pm SEM. Dosing animals with 15 μ g of the antibody causes an increase in body weight at 24 hours, 1 week and at every time point from week 3 to the completion of the study.

30

Figure 14B) Graph shows weights for the 15 μ g dosed group split into animals dosed 3 times and those dosed four times. Data expressed as means \pm 95% confidence intervals. * $P < 0.05$, repeated measures ANOVA.

35

Figure 14C) Graph shows weights for the 15 μ g dosed group split into animals dosed 3 times and those dosed four times. Compared to animals dosed with 5 μ g of the anti-NOGO antibody and animals dosed with control antibody. Data

5 expressed as means \pm 95% confidence intervals. * $P < 0.05$, repeated measures ANOVA.

Figure 15 represents the lesion volume as a percentage of total brain volume of example 11.

10

Figure 16 shows the neuroscore data represented as means \pm SEM of example 11.

15

Figures 17A, 17B and 17C. Cylinder data represented as mean \pm SEM for A) both paws, B) left paw, C) right paw of example 11.

Figure 18 shows forelimb footslips represented as mean \pm SEM of the tapered beam test of example 11.

20

Figure 19 shows hindlimb footslips represented as mean \pm SEM of example 11.

Figure 20 shows hindlimb footslips represented as mean \pm SEM (latency to cross beam test).

25 Figure 21: NOGO A transfection leads to elevation of A β 40 peptide levels in SHSY5Y-APPwt cells

Figure 22: NOGO A transfection leads to elevation of A β 42 peptide levels in SHSY5Y-APPwt cells

30

Figure 23: Effect of NOGO A expression on A β 40 peptide levels

Figure 24: Effect of NOGO A expression on A β 42 peptide levels

35 Figure 25: Effect of NOGO A, NOGO-B and NOGO-C expression on A β 40 and A β 42

5

Figure 26. Anti-NOGO A antibody 2A10-BR inhibits A β secretion from SHSY5Y-APPwt cells

Figure 27. Effect of control IgG1 on A β secretion from SHSY5Y-APPwt cells

10

Figure 28. Effect of control IgG1 on A β secretion from SHSY5Y-APPswe cells

Figure 29. Effect of control anti-NOGO (non function-blocking) antibody 6D5 on A β secretion from SHSY5Y-APPwt cells

15

Figure 30. Effect of control anti-NOGO (non function-blocking) antibody 6D5 on A β secretion from SHSY5Y-APPswe cells

20

Figure 31. Function-blocking anti-NOGO A monoclonal antibody 2A10 inhibits A β secretion from SHSY5Y-APPwt cells

Figure 32. Function-blocking anti-NOGO A monoclonal antibody 2A10 inhibits A β secretion from SHSY5Y-APPswe cells

25

Figure 33. Function-blocking anti-NOGO A monoclonal antibody 2C4 inhibits A β secretion from SHSY5Y-APPwt cells

30

Figure 34. Effect of anti-NOGO A static culture antibody preparations and additional control antibodies on A β secretion from SHSY5Y-APPwt cells. 2A10, 2C4 and 15C3 are the static culture antibodies. All others are BR (Bioreactor) purified controls or commercially available controls.

35

Figures 35A to C illustrates the dose-dependent binding of humanised antibody H1L11 in comparison with the chimera (HcLc) to human NOGO-A56 in an ELISA assay. The Y-axis shows the measured optical density (OD) at 490nm, a

- 5 quantitative measure of antibody captured in the wells. The X-axis shows the concentration of antibody used (ug/ml) per well at each data point.

Figure 36. Increased NogoA expression elevates A β levels in a dose-dependent manner. The Y axis of the graph is % increase of A β 40. The X-axis shows the increasing concentration of myc-tagged NogoA cDNA. Above the graph is a gel showing the increased amount of NogoA protein expression as shown by western blotting using an anti-NogoA antibody

15

Detailed Description of the Invention

- 20 Antibodies of the invention are typically monoclonal antibodies (mAb) and are preferably chimeric, humanised, fully human or reshaped. Of these humanised and fully human are particularly preferred.

Antibodies of the invention typically have the structure of a natural antibody or functional fragment thereof. The antibody may therefore comprise a full length antibody, a (Fab')₂ fragment, a Fab fragment, a light chain dimer or a heavy chain dimer. The antibody may be an IgG1, IgG2, IgG3, or IgG4; or IgM; IgA, IgE or IgD or a modified variant thereof. The constant domain of the antibody heavy chain may be selected accordingly. The light chain constant domain may be a kappa or lambda constant domain. Furthermore, the antibody may comprise modifications of all classes eg IgG dimers, Fc mutants that no longer bind Fc receptors or mediate Clq binding. The antibody may also be a chimeric antibody of the type described in WO86/01533 which comprises an antigen binding region and a non-immunoglobulin region. The antigen binding region is an antibody light chain variable domain or heavy chain variable domain. Typically the antigen binding region comprises both light and heavy chain variable domains. The non-immunoglobulin region is fused at its C terminus to the antigen binding region.

35

5 The non-immunoglobulin region is typically a non-immunoglobulin protein and may be an enzyme, a toxin or protein having known binding specificity. The two regions of this type of chimeric antibody may be connected via a cleavable linker sequence. Immunoadhesins having the CDRs as hereinbefore described are also contemplated in the present invention.

10 The constant region is selected according to the functionality required. Normally an IgG1 will demonstrate lytic ability through binding to complement and/or will mediate ADCC (antibody dependent cell cytotoxicity). An IgG4 will be preferred if a non-cytotoxic blocking antibody is required. However, IgG4 antibodies can demonstrate instability in production and therefore it may be more
15 preferable to modify the generally more stable IgG1. Suggested modifications are described in EP0307434 preferred modifications include at positions 235 and 237. The invention therefore provides a lytic or a non-lytic form of an antibody according to the invention.

20 In preferred forms therefore the antibody of the invention is a full length (i.e. H2L2 tetramer) non-lytic IgG1 antibody having the CDRs described supra. In most preferred forms we provide a full length non-lytic IgG1 antibody having the CDRs of SEQ ID NOs 1 to 6; SEQ ID NOs 7 to 12 or SEQ ID NOs 13 to 18.

In a further aspect, the invention provides polynucleotides encoding the
25 CDRs. For example the invention provides polynucleotides encoding CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3 as disclosed in tables 1 to 6. Preferred polynucleotide sequences are shown below in tables 7 to 12.

Table 7: Antibody 2A10/3 light chain CDRs

30

CDR	
L1	AGGTCTAGTAAGAGTCTCCTATATAAGGATG GGAAGACATACTTGAAT (SEQ ID NO:19)
L2	TTGATGTCCACCCGTGCATCA (SEQ ID NO:20)
L3	CAACAACCTTGTAAGATATCCGCTCACG (SEQ ID NO:21)

5

Table 8: Antibody 2A10/3 heavy chain CDRs

CDR	
H1	AGCTACTGGATGCAC (SEQ ID NO:22)
H2	AATATTAATCCTAGCAATGGTGGTACTAACTAC AATGAGAAGTTCAAGAGC (SEQ ID NO:23)
H3	GGACAGGGCTAC (SEQ ID NO:24)

10

Table 9: Antibody 2C4/1 light chain CDRs

15

CDR	
L1	AGATCTAGTCAGAGCCTTGTACACAGTAATG GAAACACCTATTTACAT (SEQ ID NO:25)
L2	AAAGTTTCCAACCGATTTTCT (SEQ ID NO:26)
L3	TCTCAGAGTACACATGTTCCG CTCACG (SEQ ID NO:27)

Table 10: Antibody 2C4/1 heavy chain CDRs

CDR	
H1	TTCAGTTGCTATGCCATGTCT (SEQ ID NO:28)
H2	TCCATTAGTGATGGTGGTAGTTACACCTACTAT CCAGACAATGTAAAGGGC (SEQ ID NO:29)
H3	GAACTACTTTTTGACTAC (SEQ ID NO:30)

20

5 Table 11: Antibody 15C3/3 light chain CDRs

CDR	
L1	AGGTCTAGTAAGAGTCTCCTGCATAGTAATGG CAACACTTACTTGTAT (SEQ ID NO:31)
L2	CGGATGTCCAACCTTGCCTCA (SEQ ID NO:32)
L3	ATGCAACATCTAGAATATCCGCTCACG (SEQ ID NO:33)

10

Table 12: Antibody 15C/3 heavy chain CDRs

CDR	
H1	AGCTACTGGATGAAC (SEQ ID NO:34)
H2	CAGATTTATCCTGGAGATGGTGATACTAACTAC AACGGAAAGTTCAAGGGC (SEQ ID NO:35)
H3	CGCTTTGACTAT (SEQ ID NO:36)

15 In a further aspect of the invention, there is provided a polynucleotide encoding a light chain variable region of an anti-NOGO antibody including at least one CDR selected from CDRL1, CDRL2 and CDRL3 in table 1, 3, 5, more preferably including all 3 CDRs in table 1 or all 3 CDRs in table 3 or all 3 CDRs in table 5.

20 In a further aspect of the invention, there is provided a polynucleotide encoding a heavy chain variable region of an anti-NOGO antibody including at least one CDR selected from CDRH1, CDRH2 and CDRH3, more preferably including all 3 CDRs in table 2 or all 3 CDRs in table 4 or all 3 CDRs in table 6.

5 The invention further provides an anti-NOGO antibody, or functional fragment thereof, that binds to and neutralises the activity of NOGO, preferably human NOGO and more preferably human NOGO-A which comprises a heavy chain variable region comprising one of the following amino acid sequences:-

10 QVQLQQPGTELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNINPSNGGTNY
NEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCELGGYWGQGTTTLTVSS
(SEQ ID NO: 37); or

EVQLVESGGGLVKPGGSLKLSCAASGFTFSCYAMSWVRQTPEKRLEWVASISDGGSYTTY
15 PDNVKGRFTISRDNKNNLYLQMSHLKSEDTAMYYCAKELLFDYWGQGTTTLTVSS
(SEQ ID NO: 38); or

QVQLQQSGAELVKPGASVKISCKASGYAFSSYWMNWVKQRPGKGLEWIGQIYPGDGDTNY
NGKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCAVRFDYWGQGTTTLTVSS
20 (SEQ ID NO: 39).

The invention further provides an anti-NOGO antibody, or functional fragment thereof, that binds to and neutralises NOGO which comprises a light chain variable region comprising one of the following amino acid sequences:-

25 DIVITQDELSNPVTSGESVSISCRSSKSLLYKDGKTYLNWFLQRPGQSPQLLIYLMSTRA
SGVSDRFSGSGSGTDFTLEISRVEAEDVGVYYCQQLVEYPLTFGAGTKLELK
(SEQ ID NO: 40)

30 DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNR
SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPLTFGAGTKLELK
(SEQ ID NO: 41).

35 DIVMTQAAPSPVPTPGESVSISCRSSKSLLSNGNTYLYWFLQRPGQSPQLLIYRMSNLA
SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPLTFGAGTKLELK

5 (SEQ ID NO: 42).

In a further aspect of the invention there is provided an anti-NOGO antibody, or functional fragment thereof, which binds to and neutralises the activity of NOGO, preferably human NOGO, more preferably human NOGO-A
10 which comprises:

- a) a heavy chain variable region of SEQ ID NO:37 together with a light chain variable region comprising the amino acid sequence of SEQ ID NO:40; or
- b) a heavy chain variable region of SEQ ID NO:38 together with a light chain variable region comprising the amino acid sequence of SEQ ID NO:41; or
- 15 c) a heavy chain variable region of SEQ ID NO:39 together with a light chain variable region comprising the amino acid sequence of SEQ ID NO:42.

In a further aspect of the present invention there is provided an anti-NOGO antibody, or functional fragment thereof, comprising:
20 a heavy chain variable fragment comprising SEQ ID NO:37 and a constant part or fragment thereof of a human heavy chain and
a light chain variable fragment comprising SEQ ID No:40 and a constant part or fragment thereof of a human light chain;
or
25 a heavy chain variable fragment comprising SEQ ID NO:38 and a constant part or fragment thereof of a human heavy chain; and
a light chain variable fragment comprising SEQ ID No:41 and a constant part or fragment thereof of a human light chain
or
30 a heavy chain variable fragment comprising SEQ ID NO:39 and a constant part or fragment thereof of a human heavy chain; and
a light chain variable fragment comprising SEQ ID No:42 and a constant part or fragment thereof of a human light chain.

35 In a further aspect, the invention provides polynucleotides encoding the heavy chain variable region comprising the amino acid sequences of SEQ ID

- 5 NOs 37 to 39 and light chain variable regions comprising the amino acid sequences of SEQ ID NOs 40 to 42.

A preferred polynucleotide sequence encoding the amino acid sequence of SEQ ID NO: 37 is

10

CAGGTCCAAGTGCAGCAGCCTGGGACTGAACTGGTGAAGCCTGGGGCTTCAGTGAAGCTG
TCCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTGGATGCACTGGGTGAAGCAGAGG
CCTGGACAAGGCCTTGAGTGGATTGGAAATATTAATCCTAGCAATGGTGGTACTAACTAC
AATGAGAAGTTCAAGAGCAAGGCCACACTGACTGTAGACAAATCCTCCAGCACAGCCTAC
15 ATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTATTGTGAAGTGGGACAG
GGCTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA

(SEQ ID NO: 43)

A preferred polynucleotide sequence encoding the amino acid sequence of SEQ ID NO: 38 is:

20

GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTC
CCTGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTTGCTATGCCA
TGTCTTGGGTTCGCCAGACTCCGGAAAAGAGGCTGGAGTGGGTTCGCATCC
ATTAGTGATGGTGGTAGTTACACCTACTATCCAGACAATGTAAAGGGCCG
25 ATTCACCATCTCCAGAGACAATGCCAAGAACAACCTGTACCTGCAAATGA
GCCATCTGAAGTCTGAGGACACAGCCATGTATTACTGTGCAAAGGAACTA
CTTTTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA

(SEQ ID NO: 44)

- 30 A preferred polynucleotide sequence encoding the amino acid sequence of SEQ ID NO: 39 is:

CAGGTTTCAGCTGCAGCAGTCTGGGGCTGAGCTGGTGAAGCCTGGGGCCTC
AGTGAAGATTTCTGCAAAGCTTCTGGCTACGCATTTCAGTAGCTACTGGA
TGAAGTGGGTGAAGCAGAGGCCTGGAAAGGGTCTTGAGTGGATTGGACAG
35 ATTTATCCTGGAGATGGTGATACTAACTACAACGGAAAGTTCAAGGGCAA
GGCCACACTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAGCTCA
GCAGCCTGACCTCTGAGGACTCTGCGGTCTATTTCTGTGCAGTACGCTTT

5 GACTATTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
(SEQ ID NO: 45)

A preferred polynucleotide sequence encoding the amino acid sequence of SEQ
ID NO: 40 is:

10 GATATTGTGATAACCCAGGATGAACTCTCCAATCCTGTCACTTCTGGAGA
ATCAGTTTCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTATATAAGGATG
GGAAGACATACTTGAATTGGTTTCTGCAGAGACCAGGACAATCTCCTCAG
CTCCTGATCTATTTGATGTCCACCCGTGCATCAGGAGTCTCAGACCGGTT
15 TAGTGGCAGTGGGTCAGGAACAGATTTACCCCTGGAAATCAGTAGAGTGA
AGGCTGAGGATGTGGGTGTGTATTACTGTCAACAACCTTGTAGAGTATCCG
CTCACGTTTCGGTGCTGGGACCAAGCTGGAGCTGAAA
(SEQ ID NO: 46)

20 A preferred polynucleotide sequence encoding the amino acid sequence of SEQ
ID NO: 41 is:

GATGTTGTGATGACCCAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGA
TCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCCTTGTACACAGTAATG
25 GAAACACCTATTTACATTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAAG
CTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTT
CAGTGGCAGTGGATCAGGGACAGATTTCACTCAAGATCAGCAGAGTGG
AGGCTGAGGATCTGGGAGTTTATTTCTGCTCTCAGAGTACACATGTTCCG
CTCACGTTTCGGTGCTGGGACCAAGCTGGAGCTGAAA
30 (SEQ ID NO: 47)

A preferred polynucleotide sequence encoding the amino acid sequence of SEQ
ID NO: 42 is:

35 GATATTGTGATGACTCAGGCTGCACCCTCTGTACCTGTCACTCCTGGAGA
GTCAGTATCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTGCATAGTAATG
GCAACACTTACTTGTATTGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAG

5 CTCCTGATATATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTT
 CAGTGGCAGTGGGTCAGGAAGCTGCTTTTACACTGAGAATCAGTAGAGTGG
 AGGCTGAGGATGTGGGTGTTTATTACTGTATGCAACATCTAGAATATCCG
 CTCACGTTTCGGTGCTGGGACCAAGCTGGAGCTGAAA

(SEQ ID NO: 48)

10

Anti-NOGO antibody 2A10 comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO: 37 and a light chain variable region having the amino acid sequence of SEQ ID NO: 40.

15

Anti-NOGO antibody 2C4 comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO: 38 and a light chain variable region having the amino acid sequence of SEQ ID NO: 41.

20

Anti-NOGO antibody 15C3 comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO: 39 and a light chain variable region having the amino acid sequence of SEQ ID NO: 42.

"NOGO" refers to any NOGO polypeptide, including variant forms. This includes, but is not limited to, NOGO-A having 1192 amino acid residues (GenBank accession no. AJ251383); NOGO-B, a splice variant which lacks residues 186 to 1004 in the putative extracellular domain (GenBank accession no. AJ251384) and a shorter splice variant, NOGO-C, which also lacks residues 186 to 1004 and also has smaller, alternative amino terminal domain (GenBank accession no. AJ251385) (Prinjha et al (2000) supra). All references to "NOGO" herein is understood to include any and all variant forms of NOGO such as NOGO-A and the splice variants described, unless a specific form is indicated.

"Neutralising" and grammatical variations thereof refers to inhibition, either total or partial, of NOGO function including its binding to neurones and inhibition of neurite growth.

35

"Altered antibody" refers to a protein encoded by an altered immunoglobulin coding region, which may be obtained by expression in a

5 selected host cell. Such altered antibodies include engineered antibodies (e.g., chimeric, reshaped, humanized or vectored antibodies) or antibody fragments lacking all or part of an immunoglobulin constant region, e.g., Fv, Fab, or F(ab)₂ and the like.

10 "Altered immunoglobulin coding region" refers to a nucleic acid sequence encoding altered antibody. When the altered antibody is a CDR-grafted or humanized antibody, the sequences that encode the complementarity determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner comprising human variable framework sequences. Optionally, the first immunoglobulin partner is operatively linked to a second
15 immunoglobulin partner.

20 "First immunoglobulin partner" refers to a nucleic acid sequence encoding a human framework or human immunoglobulin variable region in which the native (or naturally-occurring) CDR-encoding regions are replaced by the CDR-encoding regions of a donor antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDR regions, located within the variable region of antibodies (immunoglobulins) can be determined by known methods in the art. For example Kabat et al. (Sequences of Proteins of Immunological
25 Institutes of Health (1987)) disclose rules for locating CDRs. In addition, computer programs are known which are useful for identifying CDR regions/structures.

30 "Second immunoglobulin partner" refers to another nucleotide sequence encoding a protein or peptide to which the first immunoglobulin partner is fused in frame or by means of an optional conventional linker sequence (i.e., operatively linked). Preferably it is an immunoglobulin gene. The second immunoglobulin partner may include a nucleic acid sequence encoding the entire constant region for the same (i.e., homologous - the first and second altered antibodies are derived from the same source) or an additional (i.e., heterologous) antibody of
35 interest. It may be an immunoglobulin heavy chain or light chain (or both chains as part of a single polypeptide). The second immunoglobulin partner is not limited to a particular immunoglobulin class or isotype. In addition, the second

5 immunoglobulin partner may comprise part of an immunoglobulin constant region, such as found in a Fab, or F(ab)₂ (i.e., a discrete part of an appropriate human constant region or framework region). Such second immunoglobulin partner may also comprise a sequence encoding an integral membrane protein exposed on the outer surface of a host cell, e.g., as part of a phage display
10 library, or a sequence encoding a protein for analytical or diagnostic detection, e.g., horseradish peroxidase, β -galactosidase, etc.

The terms Fv, Fc, Fd, Fab, or F(ab)₂ are used with their standard meanings (see, e.g., Harlow et al., *Antibodies A Laboratory Manual*, Cold Spring Harbor Laboratory, (1988)).

15 As used herein, an "engineered antibody" describes a type of altered antibody, i.e., a full-length synthetic antibody (e.g., a chimeric, reshaped or humanized antibody as opposed to an antibody fragment) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts from one or more donor antibodies which have
20 specificity for the selected epitope. For example, such molecules may include antibodies characterized by a humanized heavy chain associated with an unmodified light chain (or chimeric light chain), or vice versa. Engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor antibody light and/or heavy variable domain framework
25 regions in order to retain donor antibody binding specificity. These antibodies can comprise replacement of one or more CDRs (preferably all) from the acceptor antibody with CDRs from a donor antibody described herein.

A "chimeric antibody" refers to a type of engineered antibody which contains a naturally-occurring variable region (light chain and heavy chains)
30 derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more)
35 human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., *Proc. Natl Acad Sci USA*, 86:10029-10032 (1989), Hodgson et al., *Bio/Technology*, 9:421 (1991)). A

5 suitable human acceptor antibody may be one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to
10 provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody. The prior
15 art describes several ways of producing such humanised antibodies – see for example EP-A-0239400 and EP-A-054951

"Reshaped human antibody" refers to an altered antibody in which minimally at least one CDR from a first human monoclonal donor antibody is substituted for a CDR in a second human acceptor antibody. Preferably all six
20 CDRs are replaced. More preferably an entire antigen combining region (e.g., Fv, Fab or F(ab')₂) from a first human donor monoclonal antibody is substituted for the corresponding region in a second human acceptor monoclonal antibody. Most preferably the Fab region from a first human donor is operatively linked to the appropriate constant regions of a second human acceptor antibody to form a
25 full length monoclonal antibody.

A "vectored antibody" refers to an antibody to which an agent has been attached to improve transport through the blood brain barrier (BBB). (Review see Pardridge; Advanced Drug Delivery Review 36, 299-321, 1999). The attachment may be chemical or alternatively the moiety can be engineered into the antibody.
30 One example is to make a chimera with an antibody directed towards a brain capillary endothelial cell receptor e.g. an anti-insulin receptor antibody or anti-transferrin receptor antibody (Saito et al (1995) Proc. Natl. Acad. Sci. USA 92 10227-31; Pardridge et al (1995) Pharm. Res. 12 807-816; Broadwell et al (1996) Exp. Neurol. 142 47-65; Bickel et al (1993) Proc Natl. Acad. Sci. USA 90, 2618-
35 2622; Friden et al (1996) J. Pharm. Exp. Ther. 278 1491-1498, US5182107, US5154924, US5833988, US5527527). Once bound to the receptor, both components of the bispecific antibody pass across the BBB by the process of

5 transcytosis. Alternatively the agent may be a ligand which binds such cell surface receptors eg insulin, transferrin or low density lipoprotein (Descamps et al (1996) Am. J. Physiol. 270 H1149-H1158; Duffy et al (1987) Brain Res. 420 32-38; Dehouck et al (1997) J. Cell Biol. 1997 877-889). Naturally occurring peptides such as penetratin and SynB1 and Syn B3 which are known to improve
10 transport across the BBB can also be used (Rouselle et al (2000) Mol. Pharm.57, 679-686 and Rouselle et al (2001) Journal of Pharmacology and Experimental Therapeutics 296, 124-131).

The term "donor antibody" refers to an antibody (monoclonal, and/or recombinant) which contributes the amino acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin
15 partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody.

The term "acceptor antibody" refers to an antibody (monoclonal and/or recombinant) heterologous to the donor antibody, which contributes all (or any portion, but preferably all) of the amino acid sequences encoding its heavy and/or
20 light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. Preferably a human antibody is the acceptor antibody.

25 "CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin.
30 Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate). The structure and protein folding of the antibody may mean that other residues are considered part of the antigen binding region and would be understood to be
35 so by a skilled person. See for example Chothia et al., (1989) Conformations of immunoglobulin hypervariable regions; Nature 342, p877-883. For convenience the CDR's as defined by Kabat in SEQ ID Nos 37-42 are boxed.

5 CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody
10 from which they were derived.

A "functional fragment" is a partial heavy or light chain variable sequence (e.g., minor deletions at the amino or carboxy terminus of the immunoglobulin variable region) which retains the same antigen binding specificity and the same or similar neutralizing ability as the antibody from which the fragment was
15 derived.

An "analog" is an amino acid sequence modified by at least one amino acid, wherein said modification can be chemical or a substitution or a rearrangement of a few amino acids (i.e., no more than 10), which modification permits the amino acid sequence to retain the biological characteristics, e.g.,
20 antigen specificity and high affinity, of the unmodified sequence. For example, (silent) mutations can be constructed, via substitutions, when certain endonuclease restriction sites are created within or surrounding CDR-encoding regions. The present invention contemplates the use of analogs of the antibody of the invention. It is well known that minor changes in amino acid or nucleic acid
25 sequences may lead eg to an allelic form of the original protein which retains substantially similar properties. Thus analogs of the antibody of the invention includes those in which the CDRs in the hypervariable region of the heavy and light chains are at least 80% homologous, preferably at least 90 % homologous and more preferably at least 95 % homologous to the CDRs as defined above as
30 CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3 in tables 1 to 6 and retain NOGO neutralising activity. Amino acid sequences are at least 80% homologous if they have 80% identical amino acid residues in a like position when the sequences are aligned optimally, gaps or insertions being counted as non-identical residues. The invention also contemplates analogs of the antibodies
35 of the invention wherein the framework regions are at least 80%, preferably at least 90% and more preferably at least 95% homologous to the framework regions set forth in SEQ ID NOs 37 to 42. Amino acid sequences are at least

- 5 80% homologous if they have 80% identical amino acid residues in a like position when the sequences are aligned optimally, gaps or insertions being counted as non-identical residues.

Analogous may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence. Such variations or
10 modifications may be due to degeneracy in the genetic code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

The term "effector agents" refers to non-protein carrier molecules to which
15 the altered antibodies, and/or natural or synthetic light or heavy chains of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used in the diagnostic field, e.g., polystyrene or other plastic beads, polysaccharides, e.g., as used in the BIAcore [Pharmacia] system, or other non-
20 protein substances useful in the medical field and safe for administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy metal atom, or radioisotopes. Such effector agents may also be useful to increase the half-life of the altered antibodies, e.g., polyethylene glycol.

25 Alternatively, one can construct antibodies, altered antibodies and fragments, by immunizing a non-human species (for example, bovine, ovine, monkey, chicken, rodent (e.g., murine and rat), etc.) to generate a desirable immunoglobulin upon presentation with native NOGO from any species against which antibodies cross react with human NOGO can be generated, eg human or
30 chicken. Conventional hybridoma techniques are employed to provide a hybridoma cell line secreting a non-human mAb to NOGO. Such hybridomas are then screened for binding using NOGO coated to 384- or 96-well plates, with biotinylated NOGO bound to a streptavidin coated plate or in a homogenous europium-APC linked immunoassay using biotinylated NOGO.

35 A native human antibody can be produced in a human antibody mouse such as the "Xenomouse™" (Abgenix) where the mouse immunoglobulin genes have been removed and genes encoding the human immunoglobulins have been

5 inserted into the mouse chromosome. The mice are immunised as normal and develop an antibody response that is derived from the human genes. Thus the mouse produces human antibodies obviating the need to humanize the after selection of positive hybridomas. (See Green L.L., J Immunol Methods 1999 Dec 10; 231(1-2):11-23).

10 The present invention also includes the use of Fab fragments or F(ab')₂ fragments derived from mAbs directed against NOGO. A Fab fragment contains the entire light chain and amino terminal portion of the heavy chain; and an F(ab')₂ fragment is the fragment formed by two Fab fragments bound by disulfide bonds. Fab fragments and F(ab')₂ fragments can be obtained by conventional
15 means, e.g., cleavage of mAb with the appropriate proteolytic enzymes, papain and/or pepsin, or by recombinant methods. The Fab and F(ab')₂ fragments are useful themselves as therapeutic or prophylactic, and as donors of sequences including the variable regions and CDR sequences useful in the formation of recombinant or humanized antibodies as described herein.

20 The Fab and F(ab')₂ fragments can also be constructed via a combinatorial phage library (see, e.g., Winter et al., Ann. Rev. Immunol., 12:433-455 (1994)) or via immunoglobulin chain shuffling (see, e.g., Marks et al., Bio/Technology, 10:779-783 (1992), which are both hereby incorporated by reference in their entirety.

25 Thus human antibody fragments (Fv, scFv, Fab) specific for NOGO can be isolated using human antibody fragment phage display libraries. A library of bacteriophage particles, which display the human antibody fragment proteins, are panned against the NOGO protein. Those phage displaying antibody fragments that bind the NOGO are retained from the library and clonally amplified. The
30 human antibody genes are then excised from the specific bacteriophage and inserted into human IgG expression constructs containing the human IgG constant regions to form the intact human IgG molecule with the variable regions from the isolated bacteriophage specific for NOGO.

The donor antibodies may contribute sequences, such as variable heavy
35 and/or light chain peptide sequences, framework sequences, CDR sequences, functional fragments, and analogs thereof, and the nucleic acid sequences

5 encoding them, useful in designing and obtaining various altered antibodies which are characterized by the antigen binding specificity of the donor antibody.

Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain amino acid sequences, and CDR sequences as well as functional fragments and
10 analogs thereof which share the antigen specificity of the donor antibody. Isolated nucleic acid sequences, or fragments thereof, encoding the variable chain peptide sequences or CDRs can be used to produce altered antibodies, e.g., chimeric or humanized antibodies, or other engineered antibodies when operatively combined with a second immunoglobulin partner.

15 Altered immunoglobulin molecules can encode altered antibodies which include engineered antibodies such as chimeric antibodies and humanized antibodies. A desired altered immunoglobulin coding region contains CDR-encoding regions that encode peptides having the antigen specificity of an anti-NOGO antibody, preferably a high affinity antibody, inserted into a first
20 immunoglobulin partner (a human framework or human immunoglobulin variable region).

Preferably, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner. The second immunoglobulin partner is defined above, and may include a sequence encoding a second antibody region of
25 interest, for example an Fc region. Second immunoglobulin partners may also include sequences encoding another immunoglobulin to which the light or heavy chain constant region is fused in frame or by means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of NOGO may be designed to elicit enhanced binding.

30 The second immunoglobulin partner may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the second immunoglobulin partner may be operatively linked by conventional means.

Fusion or linkage between the second immunoglobulin partners, e.g.,
35 antibody sequences, and the effector agent may be by any suitable means, e.g., by conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Such techniques

5 are known in the art and readily described in conventional chemistry and biochemistry texts.

Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the effector agent may also be constructed into the altered immunoglobulin coding
10 region. The design of such linkers is well known to those of skill in the art. In further aspects of the invention we provide diabodies (bivalent or bispecific), triabodies, tetrabodies and other multivalent scFV protein species having one or more CDRs as described supra that bind to and neutralise NOGO function.

In still a further embodiment, the antibody of the invention may have
15 attached to it an additional agent. For example, the procedure of recombinant DNA technology may be used to produce an engineered antibody of the invention in which the Fc fragment or CH₂-CH₃ domain of a full length antibody molecule has been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

20 The second immunoglobulin partner may also be operatively linked to a non-immunoglobulin peptide, protein or fragment thereof heterologous to the CDR-containing sequence having the antigen specificity of anti-NOGO antibody. The resulting protein may exhibit both anti-NOGO antigen specificity and characteristics of the non-immunoglobulin upon expression. That fusion partner
25 characteristic may be, e.g., a functional characteristic such as another binding or receptor domain, or a therapeutic characteristic if the fusion partner is itself a therapeutic protein, or additional antigenic characteristics.

Another desirable protein of this invention may comprise a full length antibody molecule, having full length heavy and light chains, or any discrete
30 fragment thereof, such as the Fab or F(ab')₂ fragments, a heavy chain dimer, or any minimal recombinant fragments thereof such as an Fv or a single-chain antibody (SCA) or any other molecule with the same specificity as the selected donor mAb. Such protein may be used in the form of an altered antibody, or may be used in its unfused form.

35 Whenever the second immunoglobulin partner is derived from an antibody different from the donor antibody, e.g. any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered

5 antibodies can comprise immunoglobulin (Ig) constant regions and variable
framework regions from one source, e.g., the acceptor antibody, and one or more
(preferably all) CDRs from the donor antibody. In addition, alterations, e.g.,
deletions, substitutions, or additions, of the acceptor mAb light and/or heavy
variable domain framework region at the nucleic acid or amino acid levels, or the
10 donor CDR regions may be made in order to retain donor antibody antigen
binding specificity.

Such engineered antibodies are designed to employ one (or both) of the
variable heavy and/or light chains of the anti-NOGO mAb or one or more of the
heavy or light chain CDRs. The engineered antibodies may be neutralising, as
15 above defined.

Such engineered antibodies may include a humanized antibody containing
the framework regions of a selected human immunoglobulin or subtype, or a
chimeric antibody containing the human heavy and light chain constant regions
fused to the anti-NOGO antibody functional fragments. A suitable human (or
20 other animal) acceptor antibody may be one selected from a conventional
database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein
database, by homology to the nucleotide and amino acid sequences of the donor
antibody. A human antibody characterized by a homology to the framework
regions of the donor antibody (on an amino acid basis) may be suitable to
25 provide a heavy chain constant region and/or a heavy chain variable framework
region for insertion of the donor CDRs. A suitable acceptor antibody capable of
donating light chain constant or variable framework regions may be selected in a
similar manner. It should be noted that the acceptor antibody heavy and light
chains are not required to originate from the same acceptor antibody.

30 Desirably the heterologous framework and constant regions are selected
from human immunoglobulin classes and isotypes, such as IgG (subtypes 1
through 4), IgM, IgA, and IgE. However, the acceptor antibody need not
comprise only human immunoglobulin protein sequences. For instance a gene
may be constructed in which a DNA sequence encoding part of a human
35 immunoglobulin chain is fused to a DNA sequence encoding a non-
immunoglobulin amino acid sequence such as a polypeptide effector or reporter
molecule.

5 Preferably, in a humanized antibody, the variable domains in both human heavy and light chains have been engineered by one or more CDR replacements. It is possible to use all six CDRs, or various combinations of less than the six CDRs. Preferably all six CDRs are replaced. It is possible to replace the CDRs only in the human heavy chain, using as light chain the unmodified
10 light chain from the human acceptor antibody. Alternatively, a compatible light chain may be selected from another human antibody by recourse to the conventional antibody databases. The remainder of the engineered antibody may be derived from any suitable acceptor human immunoglobulin.

The engineered humanized antibody thus preferably has the structure of a
15 natural human antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use.

It will be understood by those skilled in the art that an engineered antibody may be further modified by changes in variable domain amino acids without necessarily affecting the specificity and high affinity of the donor antibody (i.e., an
20 analog). It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both.

In addition, the constant region may be altered to enhance or decrease selective properties of the molecules of the instant invention. For example,
25 dimerization, binding to Fc receptors, or the ability to bind and activate complement (see, e.g., Angal et al., Mol. Immunol, 30:105-108 (1993), Xu et al., J. Biol. Chem, 269:3469-3474 (1994), Winter et al., EP 307,434-B).

An altered antibody which is a chimeric antibody differs from the humanized antibodies described above by providing the entire non-human donor
30 antibody heavy chain and light chain variable regions, including framework regions, in association with immunoglobulin constant regions from other species, preferably human for both chains.

Preferably, the variable light and/or heavy chain sequences and the CDRs of suitable donor mAbs, and their encoding nucleic acid sequences, are utilized
35 in the construction of altered antibodies, preferably humanized antibodies, of this invention, by the following process. The same or similar techniques may also be employed to generate other embodiments of this invention.

5 A hybridoma producing a selected donor mAb is conventionally cloned, and the DNA of its heavy and light chain variable regions obtained by techniques known to one of skill in the art, e.g., the techniques described in Sambrook et al., (Molecular Cloning (A Laboratory Manual), 2nd edition, Cold Spring Harbor Laboratory (1989)). The variable heavy and light regions containing at least the
10 CDR-encoding regions and those portions of the acceptor mAb light and/or heavy variable domain framework regions required in order to retain donor mAb binding specificity, as well as the remaining immunoglobulin-derived parts of the antibody chain derived from a human immunoglobulin are obtained using polynucleotide primers and reverse transcriptase. The CDR-encoding regions are identified
15 using a known database and by comparison to other antibodies.

 A mouse/human chimeric antibody may then be prepared and assayed for binding ability. Such a chimeric antibody contains the entire non-human donor antibody VH and VL regions, in association with human Ig constant regions for both chains.

20 Homologous framework regions of a heavy chain variable region from a human antibody may be identified using computerized databases, e.g., KABAT®, and a human antibody having homology to the donor antibody will be selected as the acceptor antibody. A suitable light chain variable framework region can be designed in a similar manner.

25 A humanized antibody may be derived from the chimeric antibody, or preferably, made synthetically by inserting the donor mAb CDR-encoding regions from the heavy and light chains appropriately within the selected heavy and light chain framework. Alternatively, a humanized antibody can be made using standard mutagenesis techniques. Thus, the resulting humanized antibody
30 contains human framework regions and donor mAb CDR-encoding regions. There may be subsequent manipulation of framework residues. The resulting humanized antibody can be expressed in recombinant host cells, e.g., COS, CHO or myeloma cells.

 A conventional expression vector or recombinant plasmid is produced by
35 placing these coding sequences for the antibody in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences

5 include promoter sequences, e.g., CMV promoter, and signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antibody light or heavy chain. Preferably this second expression vector is identical to the first except insofar as the coding sequences and
10 selectable markers are concerned, so to ensure as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the altered antibody may reside on a single vector.

A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create
15 the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The antibody which includes the association of both the recombinant heavy chain and/or light chain is screened from culture by appropriate assay, such as ELISA
20 or RIA. Similar conventional techniques may be employed to construct other altered antibodies and molecules.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning
25 vectors may be used. One vector, pUC19, is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated may be used for cloning. Thus, the
30 selection of the cloning vector is not a limiting factor in this invention.

Similarly, the vectors employed for expression of the antibodies may be selected by one of skill in the art from any conventional vector. The vectors also contain selected regulatory sequences (such as CMV or RSV promoters) which direct the replication and expression of heterologous DNA sequences in selected
35 host cells. These vectors contain the above described DNA sequences which code for the antibody or altered immunoglobulin coding region. In addition, the

5 vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences
10 include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

The components of such vectors, e.g. replicons, selection genes,
15 enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal
20 expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the antibodies or altered immunoglobulin molecules thereof. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However,
25 most desirably, cells from various strains of E. coli are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this invention.

Suitable host cells or cell lines for the expression of the antibody of the invention are preferably mammalian cells such as NS0, Sp2/0, CHO (e.g. DG44),
30 COS, a fibroblast cell (e.g., 3T3), and myeloma cells, and more preferably a CHO or a myeloma cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product
35 production and purification are known in the art. See, e.g., Sambrook et al., cited above.

5 Bacterial cells may prove useful as host cells suitable for the expression of the recombinant Fabs of the present invention (see, e.g., Plückthun, A., Immunol. Rev., 130:151-188 (1992)). However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant Fab produced in a bacterial cell would have
10 to be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of E. coli used for expression are well-known as host cells in the field of biotechnology. Various strains of B. subtilis, Streptomyces, other bacilli and the like may also be
15 employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. Drosophila and Lepidoptera and viral expression systems. See, e.g. Miller et al., Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein.

20 The general methods by which the vectors may be constructed, the transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the antibody of the invention from such host cell are all conventional techniques. Typically, the culture method of the present invention is a serum-free culture method, usually by culturing cells
25 serum-free in suspension. Likewise, once produced, the antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention. For example, preparation
30 of altered antibodies are described in WO 99/58679 and WO 96/16990.

Yet another method of expression of the antibodies may utilize expression in a transgenic animal, such as described in U. S. Patent No. 4,873,316. This relates to an expression system using the animal's casein promoter which when transgenically incorporated into a mammal permits the female to produce the
35 desired recombinant protein in its milk.

In a further aspect of the invention there is provided a method of producing an antibody of the invention which method comprises the step of culturing a host

- 5 cell transformed or transfected with a vector encoding the light and/or heavy chain of the antibody of the invention and recovering the antibody thereby produced.

In accordance with the present invention there is provided a method of producing an anti-NOGO antibody which specifically binds to and neutralises the activity of human NOGO-A which method comprises the steps of;

- 10 (a) providing a first vector encoding a heavy chain of the antibody;
- (b) providing a second vector encoding the light chain of the antibody;
- (c) transforming a mammalian host cell (e.g. CHO) with said first and second vectors;
- 15 (d) culturing the host cell of step (c) under conditions conducive to the secretion of the antibody from said host cell into said culture media;
- (e) recovering the secreted antibody of step (d).

Once expressed by the desired method, the antibody is then examined for in vitro activity by use of an appropriate assay. Presently conventional ELISA assay formats are employed to assess qualitative and quantitative binding of the antibody to NOGO. Additionally, other in vitro assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to evaluate the persistence of the antibody in the body despite the usual clearance mechanisms.

The therapeutic agents of this invention may be administered as a prophylactic or following the stroke event/on-set of clinical symptoms, or as otherwise needed. The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient. It is envisaged that repeated dosing (e.g. once a week or once every two weeks) over an extended time period (e.g. four to six months) maybe required to achieve maximal therapeutic efficacy.

The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the host. The antagonists and antibodies, and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intrathecally,

- 5 intraperitoneally, intramuscularly, intravenously, or intranasally, of which intravenously is particularly preferred.

Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the antagonist or antibody of the invention as an active ingredient in a pharmaceutically acceptable carrier. In the
10 prophylactic agent of the invention, an aqueous suspension or solution containing the engineered antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the antagonist or antibody of the invention or a cocktail thereof dissolved in a pharmaceutically acceptable carrier, preferably an
15 aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.9% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate
20 physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antagonist or antibody of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the
25 particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of an antagonist or antibody of the invention.
30 Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 30 and preferably 5 mg to about 25 mg of an engineered antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are
35 described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania. For the preparation of intravenously administrable antibody formulations of the invention see Lasmar

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Ha,E Wang W, Wang Y.j. "Peroxide formation in polysorbate 80 and protein stability", J. Pharm Sci, 91, 2252-2264,(2002) the entire contents of which are incorporated herein by reference and to which the reader is specifically
20 referred.

It is preferred that the therapeutic agent of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. To effectively treat stroke and other neurological diseases in a human, one
25 dose of up to 700 mg per 70 kg body weight of an antagonist or antibody of this invention should be administered parenterally, preferably i.v. or i.m. (intramuscularly). Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician. As disclosed in the examples, the present inventors have been able to demonstrate a positive effect on
30 functional recovery in the rat model therein when antibodies of the invention were administered intravenously.

The antibodies described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and
35 reconstitution techniques can be employed.

5 Antibodies of the invention may also be used in combination (i.e. simultaneously, sequentially or separately) with a neurotrophic factor such as nerve growth factor (NGF), for example brain derived neurotrophic factor (BDNF), anti-inflammatory agents such as corticosteroids, and/or tPA. Combinations of a NOGO antibody of the invention and e.g. tPA maybe assessed in the MCAO
10 model set forth in the examples below.

 In another aspect, the invention provides a pharmaceutical composition comprising anti-NOGO antibody of the present invention or a functional fragment thereof and a pharmaceutically acceptable carrier for treatment or prophylaxis of
15 stroke and other neurological diseases.

 In a yet further aspect, the invention provides a pharmaceutical composition comprising the anti-NOGO antibody of the present invention or a functional fragment thereof and a pharmaceutically acceptable carrier for inhibiting neurodegeneration and/or promoting functional recovery in a human
20 patient suffering, or at risk of developing, a stroke or other neurological disease.

 The invention further provides a method of treatment or prophylaxis of stroke (particularly ischemic stroke) and other neurological diseases/disorders, in particular Alzheimer's disease, in a human which comprises administering to said human in need thereof an effective amount of an anti-NOGO antibody or a
25 functional fragment thereof. Antibodies of the invention may be used in methods of treatment to slow or halt the progression and/or onset of Alzheimer's disease in addition to (or as an alternative to) treating established disease in a human patient.

 Further the invention provides the use of an anti-NOGO antibody, or a
30 functional fragment thereof, in the preparation of a medicament for treatment or prophylaxis of stroke and other neurological diseases/disorders, in particular Alzheimer's disease.

 The invention also provides a method of inhibiting neurodegeneration and/or promoting functional recovery in a human patient suffering, or at risk of
35 developing, a stroke or other neurological disease/disorder, in particular Alzheimer's disease, which comprises administering to said human in need

5 thereof an effective amount of an anti-NOGO antibody or a functional fragment thereof.

In addition the invention provides the use of an anti-NOGO antibody or a functional fragment thereof in the preparation of a medicament for inhibiting neurodegeneration and/or promoting functional recovery in a human patient
10 afflicted with, or at risk of developing, a stroke and other neurological disease/disorder, in particular Alzheimer's disease.

The invention further provides a method of treating or prophylaxis of stroke or other neurological disease/disorder, in particular Alzheimer's disease, in a human comprising the step of parenteral administration of a therapeutically
15 effective amount of an anti-NOGO antibody. Preferably the anti-NOGO antibody is administered intravenously.

Neurological diseases or disorders as used hereinabove includes, but is not limited to traumatic brain injury, spinal cord injury, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease,
20 multiple sclerosis and in particular Alzheimer's disease.

The invention also provides a method of promoting axonal sprouting comprising the step of contacting a human axon with an anti-NOGO antibody. This method may be performed in-vitro or in-vivo, preferably the method is performed in-vivo.
25

In a further aspect therefore there is provided the use of an anti-NOGO antibody or functional fragment thereof of the invention comprising CDR's of table 1 and 2; CDR's of Table 3 and 4; or CDR's of table 5 and 6 in intravenously administrable form in the manufacture of a medicament for the treatment of stroke (particularly
30 ischemic stroke), brain injury, spinal cord injury, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease, multiple sclerosis and in particular Alzheimer's disease in a human patient.

In a further aspect therefore there is provided a method of treating stroke (particularly ischemic stroke), brain injury, spinal cord injury, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease,
35 Huntington's disease, multiple sclerosis and in particular Alzheimer's disease in a

- 5 human patient which method comprises the intravenous administration of a therapeutically effective amount of an anti-NOGO antibody of the invention.

In a further aspect of the present invention there is provided a method of promoting axon sprouting of neurons within the central nervous system of a
10 human subject (e.g. patient) which method comprises administering (e.g. intravenously administering) a therapeutically effective amount of an anti-NOGO antibody (e.g. an anti-NOGO antibody comprising CDRs as set forth herein).

In a further aspect of the present invention there is provided the use of an anti-
15 NOGO antibody (e.g. an anti-NOGO antibody comprising the CDRs set forth herein) in the manufacture of an intravenously administrable medicament for the treatment of stroke (particularly ischemic stroke), brain injury, spinal cord injury, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease, multiple sclerosis and in particular Alzheimer's
20 disease in a human patient.

In a further aspect of the invention there is provided a method of regenerating axon processes in neurons of the central nervous system in a human patient afflicted with (or susceptible to) stroke (particularly ischemic stroke), brain injury,
25 spinal cord injury, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease, multiple sclerosis and in particular Alzheimer's disease which method comprises the step of administering (e.g. intravenously) a therapeutically effective amount of an anti-NOGO antibody (e.g. an anti-NOGO antibody having the CDRs set forth herein).

30

In a further aspect of the invention there is provided the use of an anti-NOGO antibody (e.g. an anti-NOGO antibody having the CDRs set forth herein) in the manufacture of an intravenously administrable pharmaceutical composition for regenerating axon processes in neurons of the central nervous system in a
35 human patient afflicted with (or susceptible to) stroke (particularly ischemic stroke), brain injury, spinal cord injury, fronto-temporal dementias (tauopathies),

- 5 peripheral neuropathy, Parkinson's disease, Huntington's disease, multiple sclerosis and in particular Alzheimer's disease.

In a further aspect of the invention there is provided a method of modulating the production of an amyloidogenic peptide comprising contacting a cell which is
10 expressing the precursor from which the amyloidogenic peptide is derived and a NOGO polypeptide (e.g. human NOGO-A) with an anti-NOGO antibody (e.g. an anti-NOGO antibody comprising the CDRs set forth herein, particularly 2A10 and fully human or humanised versions thereof). In typical embodiments, the precursor is APP. In further typical embodiments the amyloidogenic peptide is
15 A β , most preferably A β 40, A β 42 or a combination of both.

As used herein, the term "functional recovery" refers to a motor and/or sensory and/or behavioural improvement in a subject following e.g. an ischemic event or
20 injury or on-set of clinical symptoms. Functional recovery in humans may be evaluated by instruments designed to measure elemental neurological functions such as motor strength, sensation and coordination, cognitive functions such as memory, language and the ability to follow directions, and functional capacities such as basic activities of daily living or instrumental activities. Recovery of
25 elemental neurological function can be measured with instruments such as the NIH Stroke Scale (NIHSS), recovery of cognitive function can be measured with neuropsychological tests such as Boston Naming Test, Trail-making Tests, and California Verbal Learning Test, and activities of daily living may be measured with instruments such as the ADCS/ADL (Alzheimer's Disease Clinical
30 Studies/Activities of Daily Living) scale or the Bristol Activities of Daily Living Scale, all tests and scales known in the art.

5 The following examples illustrate but do not limit the invention.

Exemplification.

10

Example 1 – Preparation and selection of the hybridomas

Anti-NOGO monoclonal antibodies are produced by hybridoma cells, the result of the fusion of mouse myeloma cells with B lymphocytes from mice
15 immunised with the target antigen. The hybridoma cell is immortalised by the myeloma fusion partner while the capacity to produce antibodies is provided by the B lymphocyte. Each hybridoma cell makes only one individual antibody with unique specificity hence the term monoclonal.

SJL mice were immunised with 10µg total protein (1:1, human NOGO-A splice (amino acids 186-1004) and rat NOGO-A splice (amino acids 173-975),
20 produced as GST-fusion proteins in E.Coli BL21) using both CFA and RIBI adjuvants subcutaneously. The mice were then boosted with 5µg of the same proteins using RIBI adjuvant after 4 and 8 days. After a further 3 days, immune cells were harvested from the locally draining lymph nodes and fused with mouse
25 myeloma cells using PEG1500 to generate hybridomas. Individual hybridoma cell lines were cloned by two rounds of limiting dilution. By immunising the mice with both human and rat NOGO-A, antibodies may be raised that have good binding specificity and/or binding affinity for both rat as well as human NOGO-A. This in turn enables evaluation of such antibodies in rat and/or rodent models prior to
30 administration to a human.

Initial hybridoma antibody selection was on the basis of direct binding to the NOGO protein(s) on microtitre plates. Subsequently approximately 60 hybridomas were selected based on the ability of soluble protein (consisting of human NOGO-A sequence cleaved from the GST moiety using Prescission™
35 protease) to compete for this binding activity in ELISA assays.

5

Example 2 – Cloning of the variable regions

Total RNA was extracted from the selected 2A10/3, 2C4/1 and 15C3/3 hybridoma cells followed by reverse transcription and polymerase chain reaction (RT-PCR) to extract heavy and light variable-domain cDNA sequence. The forward primer for RT-PCR was a mixture of degenerate primers specific for murine immunoglobulin gene leader-sequences and the reverse primer was an isotype-specific antibody directed to the constant regions. PCR primers were designed to carry 5' restriction enzyme recognition sites to enable cloning into pUC19 for DNA sequencing.

15

RNA extraction

Total RNA was extracted from pellets of 10^6 cells of each hybridoma clone using the SV Total RNA Isolation System from Promega according to manufacturer's instructions.

20

Reverse transcription

RNA was reverse transcribed to produce cDNA of the variable heavy and light domains using forward primers specific for the murine leader sequences and reverse primers to murine IgGk constant regions. The IgGy1 reverse primer was used for hybridomas 2C4/1 and 15C3/3; and the IgGy2b for 2A10/3. Forward primers carry a Sall restriction enzyme recognition site at the 5' end, with four extra nucleotides added 5'to this for efficient restriction digestion. These primers were adapted from Jones ST and Bendig MM 1991 (Biotechnology 9, 88-89). Reverse primers carry a XmaI restriction enzyme recognition site plus and extra four nucleotides at the 5' ends.

30

Primers:

Murine V_H leader sequence forward primers:

AG77: 5'-ACT AGT CGA CAT GAA ATG CAG CTG GGT CAT STT CTT C-3'
(SEQ.I.D.NO:51)

35

AG78: 5'-ACT AGT CGA CAT GGG ATG GAG CTR TAT CAT SYT CTT-3'
(SEQ.I.D.NO:52)

- 5 AG79: 5'-ACT AGT CGA CAT GAA GWT GTG GTT AAA CTG GGT TTT T-3'
(SEQ.I.D.NO:53)
AG80: 5'-ACT AGT CGA CAT GRA CTT TGG GYT CAG CTT GRT TT-3'
(SEQ.I.D.NO:54)
AG81: 5'-ACT AGT CGA CAT GGA CTC CAG GCT CAA TTT AGT TTT CCT T-
10 3' (SEQ.I.D.NO:55)
AG82: 5'-ACT AGT CGA CAT GGC TGT CYT RGS GCT RCT CTT CTG C-3'
(SEQ.I.D.NO:56)
AG83: 5'-ACT AGT CGA CAT GGR ATG GAG CKG GRT CTT TMT CTT-3'
(SEQ.I.D.NO:57)
15 AG84: 5'-ACT AGT CGA CAT GAG AGT GCT GAT TCT TTT GTG-3'
(SEQ.I.D.NO:58)
AG85: 5'-ACT AGT CGA CAT GGM TTG GGT GTG GAM CTT GCT ATT CCT G-3'
(SEQ.I.D.NO:59)
AG86: 5'-ACT AGT CGA CAT GGG CAG ACT TAC ATT CTC ATT CCT G-3'
20 (SEQ.I.D.NO:60)
AG87: 5'-ACT AGT CGA CAT GGA TTT TGG GCT GAT TTT TTT TAT TG-3'
(SEQ.I.D.NO:61)
AG89: 5'-ACT AGT CGA CAT GAT GGT GTT AAG TCT TCT GTA CCT G-3'
(SEQ.I.D.NO:62)
25
Murine V_L leader sequence forward primers:
AG90: 5'-ACT AGT CGA CAT GAA GTT GCC TGT TAG GCT GTT GGT GCT G-
3' (SEQ.I.D.NO:63)
AG91: 5'-ACT AGT CGA CAT GGA GWC AGA CAC ACT CCT GYT ATG GGT-3'
30 (SEQ.I.D.NO:64)
AG92: 5'-ACT AGT CGA CAT GAG TGT GCT CAC TCA GGT CCT GGC GTT G-3'
(SEQ.I.D.NO:65)
AG93: 5'-ACT AGT CGA CAT GAG GRC CCC TGC TCA GWT TYT TGG MWT
CTT G-3' (SEQ.I.D.NO:66)
35 AG94: 5'-ACT AGT CGA CAT GGA TTT WCA GGT GCA GAT TWT CAG CTT C-3'
(SEQ.I.D.NO:67)

- 5 AG95: 5'-ACT AGT CGA CAT GAG GTK CYY TGY TSA GYT YCT GRG G-3'
(SEQ.I.D.NO:68)
AG96: 5'-ACT AGT CGA CAT GGG CWT CAA GAT GGA GTC ACA KWy YCW
GG-3' (SEQ.I.D.NO:69)
AG97: 5'-ACT AGT CGA CAT GTG GGG AYC TKT TTY CMM TTT TTC AAT
10 TG-3' (SEQ.I.D.NO:70)
AG98: 5'-ACT AGT CGA CAT GGT RTC CWC ASC TCA GTT CCT TG-3'
(SEQ.I.D.NO:71)
AG99: 5'-ACT AGT CGA CAT GTA TAT ATG TTT GTT GTC TAT TTC T-3'
(SEQ.I.D.NO:72)
15 AG100: 5'-ACT AGT CGA CAT GGA AGC CCC AGC TCA GCT TCT CTT CC-3'
(SEQ.I.D.NO:73)
MKV12: 5'-ACT AGT CGA CAT GAA GTT TCC TTC TCA ACT TCT GCT C-3'
(SEQ.I.D.NO:74)
20 Murine y1 constant region reverse primer:
AG102: 5'-GGA TCC CGG GCC AGT GGA TAG ACA GAT G-3' (SEQ.I.D.NO:75)
Murine y2b constant region reverse primer:
AG104: 5'-GGA TCC CGG GAG TGG ATA GAC TGA TGG-3' (SEQ.I.D.NO:76)
25 Murine k constant region reverse primer:
AG101: 5'-GGA TCC CGG GTG GAT GGT GGG AAG ATG-3' (SEQ.I.D.NO:77)

30 Pools of murine V_H or V_L leader sequence forward primers were prepared at
50µM. Solutions of the murine γ or κ constant region reverse primers were also
prepared at 50µM.

5 Reverse Transcriptase PCR (RT-PCR).

Reverse transcription of the RNA encoding the variable heavy and light regions was carried out in duplicate using the Access RT-PCR System from Promega according to manufacturer's instructions. Approximately 200ng RNA was included in a 50 µl reaction containing RT-PCR buffer supplied, 0.2 mM dNTPs, 10 1 µM of each primer set, 1 µM MgSO₄ and 5U each of AMV Reverse transcriptase and Tfl DNA polymerase.

RT-PCR cycle: 1- 48°C for 45min
 2- 94°C for 2min
 3- 94°C for 30sec
15 4- 50°C for 1min
 5- 68°C for 2min
 6- 68°C for 7min

steps 3 to 5: repeat 30 times.

20 pUC19 cloning

The variable region RT-PCR products were purified using a Qiagen MinElute Qiagen PCR Purification kit according to their instructions and digested sequentially with XmaI and Sall from New England Biolabs according to manufacture's instructions. They were then loaded on a preparative 1% agarose 25 gel containing 0.5% ethidium bromide and run in TAE buffer at 50mA for 1hour and the V region bands excised under ultra-violet light. The DNA fragments were purified from the gel using the MinElute Gel extraction kit from Qiagen according to manufacturer's instructions. pUC19 vector arms were prepared by digesting pUC19 with Sall and XmaI, then purified using the MinElute Reaction Clean up 30 kit from Qiagen and dephosphorylated using Shrimp alkaline phosphatase (USB) according to the manufacturer's instructions. The concentration of the vector arms and the V-region fragments was estimated from an analytical 1% agarose/ethidium bromide gel, mixed in a molar ratio of 1:2 and ligated using Promega's Quick Ligation kit according to the manufacturer's instructions.

35 Ligated plasmids were transformed into DH5α cells (Invitrogen) according manufacturer's instructions. Colonies which grew on L-agar plates containing 100µg/ml ampicillin were selected for DNA sequence analysis.

5

Variable region sequencing

Colonies were cultured overnight at 37°C in 5ml LB medium supplemented with 100µg/ml ampicillin and plasmid DNA was extracted and purified using the Qiagen QIAprep Spin Miniprep kit according to manufacturer's instructions. The V_H and V_L regions were DNA sequenced using standard M13 forward and reverse primers.

The results of the sequencing determination are shown as SEQ ID NOs 43 to 48.

Example 3 – Recombinant anti-NOGO antibodies

15

Recombinant antibodies having murine 2a/k constant regions could be purified from cells transfected with plasmids comprising the light and heavy variable regions cloned onto mouse IgG2a/k constant region gene segments. The cloned murine V regions were amplified by PCR to introduce restriction sites required for cloning into mammalian expression vectors Rld and Rln. Hind III and Spe I sites were designed in frame with the V_H domain to allow cloning into a modified Rld vector containing the mouse γ2a constant region. Hind III and BsiW I sites were designed in frame the V_L domain and allow cloning into a modified Rln vector containing the mouse κ constant region.

25

PCR primers2A10 V_H forward primer:

5'- ACTCATAAGCTTGCCACCATGGGATGGAGCTGTATCATCCTCTTTTGGTAG -3'

(SEQ.I.D.NO:78)

30

V_H reverse primer:

5'-ACTATGACTAGTGTGCCTTGGCCCCAGTAG-3' (SEQ.I.D.NO:79)

V_L forward primer:

5'- ACTCATAAGCTTGCCACCATGAGGTGCTCTCTTCAGTTTCTG -3' (SEQ.I.D.NO:80)

V_L reverse primer:

35

5'- ACTATGCGTACGTTTCAGCTCCAGCTTGG -3' (SEQ.I.D.NO:81)

- 5 PCR was performed using Hercules (Stratagene) according to the manufacturer's instructions in 50µl volume containing approx 10ng of the pUC19 miniprep containing the V-region, 2% DMSO, 400µM dNTPs, 1µM each primer and buffer supplied. PCR was carried out as follows 1-95 °C 2 mins, 2-95°C 1 min, 3-56 °C 1 min, 4-72°C 1 min.. Steps 2-4 30 cycles.

10

Cloning into expression vectors

- The PCR products were purified using the MinElute PCR Purification kit from Qiagen according to manufacturer's instructions. The V_H PCR product and Rld (IgG2a) mammalian expression vector were digested Hind III-Spe I. The V_L PCR product and Rln (k) mammalian expression vector were digested Hind III-BsiW I (NEB) according to manufacturer's instructions. Vectors were ligated to inserts in a 1:2 molar ratio using the Promega Quick Ligation kit. Ligation mixes were transfected into DH5α cells and colonies growing on ampicillin selection were grown up and sent for DNA sequence verification.

20

Sequencing of recombinant anti-NOGO antibody 2A10/3

The sequence of the 2A10 heavy chain between the HindIII and EcoRI cloning sites was determined to be:

- 25 AAGCTTGCCACCATGGGATGGAGCTGTATCATCCTCTTTTGGTAGCAGC
AGCTACAGGTGTCCACTCCCAGGTCCAAGTGCAGCAGCCTGGGACTGAAC
TGGTGAAGCCTGGGGCTTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTAC
ACCTTCACCAGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGG
CCTTGAGTGGATTGGAAATATTAATCCTAGCAATGGTGGTACTAACTACA
30 ATGAGAAGTTCAAGAGCAAGGCCACACTGACTGTAGACAAATCCTCCAGC
ACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTA
TTATTGTGAAGTGGGACAGGGCTACTGGGGCCAAGGCACACTAGTCACCG
TCTCCTCAGCCAAAACAACAGCCCCATCGGTCTATCCACTGGCCCCCTGTG
TGTGGAGATACTGGCTCCTCGGTGACTCTAGGATGCCTGGTCAAGGG
35 TTATTTCCCTGAGCCAGTGACCTTGACCTGGAACTCTGGATCCCTGTCCA
GTGGTGTGCACACCTTCCCAGCTGTCTGACGTCTGACCTCTACACCTC
AGCAGCTCAGTGACTGTAACCTCGAGCACCTGGCCCAGCCAGTCCATCAC

5 CTGCAATGTGGCCCAACCGGCAAGCAGCACCAAGGTGGACAAGAAAATTG
 AGCCCAGAGGGCCCACAATCAAGCCCTGTCCTCCATGCAAATGCCCAGCA
 CCTAACCTCCTGGGTGGCCCATCCGTCTTCATCTTCCCTCCAAAGATCAA
 GGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGG
 ATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAAC
 10 GTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAGGATTACAACAG
 TACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACCAGGACTGGATGA
 GTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCCCAGCGCCC
 ATCGAGAGAACCATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACAGGT
 ATATGTCTTGCCCTCCACCAGAAGAAGAGATGACTAAGAAACAGGTCACTC
 15 TGACCTGCATGGTCACAGACTTCATGCCTGAAGACATTTACGTGGAGTGG
 ACCAACAACGGGAAAACAGAGCTAAACTACAAGAACACTGAACCAGTCCCT
 GGACTCTGATGGTTCTTACTTCATGTACAGCAAGCTGAGAGTGGAAGA
 AGAACTGGGTGGAAAGAAATAGCTACTCCTGTTCAGTGGTCCACGAGGGT
 CTGCACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCGGGTAAATG
 20 AGAATTC
 (SEQ ID NO: 49)

The sequence of the 2A10 light chain between the HindIII and EcoRI cloning sites was determined to be:

25 AAGCTTGCCACCATGAGGTGCTCTCTTCAGTTTCTGGGGGTGCTTATGTT
 CTGGATCTCTGGAGTCAGTGGGGATATTGTGATAACCCAGGATGAACTCT
 CCAATCCTGTCACTTCTGGAGAATCAGTTTCCATCTCCTGCAGGTCTAGT
 AAGAGTCTCCTATATAAGGATGGGAAGACATACTTGAATTGGTTTCTGCA
 GAGACCAGGACAATCTCCTCAGCTCCTGATCTATTTGATGTCCACCCGTG
 30 CATCAGGAGTCTCAGACCGGTTTAGTGGCAGTGGGTGAGGAACAGATTTT
 ACCCTGGAAATCAGTAGAGTGAAGGCTGAGGATGTGGGTGTGTATTACTG
 TCAACAACCTTGTAGAGTATCCGCTCACGTTCCGGTGCTGGGACCAAGCTGG
 AGCTGAAACGTACGGATGCTGCACCGACTGTATCCATCTTCCCACCATCC
 AGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAA
 35 CTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAAC
 GACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGC
 ACCTACAGCATGAGCAGCACCCCTCACGTTGACCAAGGACGAGTATGAACG

5 ACATAACAGCTATACCTGTGAGGCCACTCACAAAGACATCAACTTCACCCA
 TTGTCAAGAGCTTCAACAGGAATGAGTGTTAAGAATTC
 (SEQ ID NO: 50)

A chimaera of 2A10 was also constructed as is referred to herein as HcLc.

10

Example 4 – Mouse anti-NOGO antibody binds to NOGO

GST-human NOGO-A56 at 5µg/ml in 50mM Tris pH9.5 was coated onto Nunc Immunosorp plates (100µl per well) at 4 °C overnight. Wells were rinsed once with PBS then incubated with 1% BSA in PBS to block non-specific binding sites at room temperature for 1hour. Antibodies were diluted in PBS to 2µg/ml and 1/3 dilutions made from this. Antibodies were added to wells in triplicate and incubated at 4C overnight. Wells were washed three times with PBS then incubated with Anti-Mouse-HRP (1:1000) for 1hour. Washed five times with PBS and then incubated with 100µl TMB substrate (Sigma) per well for 10 minutes.

15 The colour reaction was stopped by the addition of 50µl concentrated HCl. Optical density at 450nm was measured using a plate reader. Background values read from wells with no antibody were subtracted.

Figures 8 shows the dose-dependent binding of all three mouse anti-NOGO-A monoclonal antibodies, 2A10, 2C4 and 15C3, to human NOGO-A56 in an ELISA assay.

The Y-axis shows the measured optical density (OD) at 450nm, a quantitative measure of antibody captured in the wells. The X-axis shows the concentration of antibody used (ng/ml) per well at each data point. Antibody 2A10 shows the highest signal at a range of concentrations suggestive of a higher affinity for human NOGO-A.

25

30

Example 5 – Production of Inhibitory NOGO-A Fragment (NOGO-A56, SEQ.I.D.NO:87)

35 A cDNA sequence encoding amino acids 586-785

(MQESLYPAAQLCPSFEESEATPSPVLPDIVMEAPLNSAVPSAGASVIQPS SSPLEASSV
 NYESIKHEPENPPPYYEAMS VSLKKVSGIKKEIKEPENINAALQETEAPYIS IACDLIKE

5 TKLSAEPAPDFSDYSEMAKVEQVPDPHSELVEDSSPDSEPVDLFSDDSI PDVPQKQDETV
MLVKESLTETSFESMIEYENKE – SEQ.I.D.NO:87) of human NOGO-A was cloned
into the BamHI-XhoI sites of pGEX-6P1 to generate a GST-tagged fusion protein
designated NOGO-A56. Plasmid was expressed in BL21 cells in 2XTY medium
with 100µg/ml ampicillin following induction with IPTG to 0.5mM at 37C for
10 3hours. Cell pellets were lysed by sonication and the fusion protein purified using
Glutathione-sepharose (Amersham Pharmacia) following manufacturers
instructions. Purified protein was eluted using reduced glutathione and
extensively dialysed against PBS, quantitated using BSA standards and a
BioRad coomassie based protein assay and then stored in aliquots at -80C.
15 Thus the present invention provides an antibody or functional fragment thereof
which binds to NOGO-A, particularly human NOGO-A wherein said antibody or
functional fragment thereof neutralises the activity of a NOGO protein comprising
the polypeptide encoded by SEQ.I.D.NO:87 wherein said antibody binds to
SEQ.I.D.NO:87 of said protein. In typical forms, antibodies of the invention bind
20 between amino acids 586-785 of human NOGO-A and neutralise the activity of
NOGO-A.

Example 6 - Neurite-outgrowth Assay

25 Control GST only or GST-NOGOA56 fusion proteins were thawed on ice and
diluted in 0.5x tissue culture grade PBS to 3pmol/µl. 5µl spots were dried onto the
centre of each well of BD-Biocoat poly-d-lysine coated 96 well plates in the
tissue-culture cabinet. Once dried, purified antibodies, hybridoma conditioned
30 tissue-culture supernatant or compounds were diluted in HBSS (Life
Technologies) and 50µl applied to wells in replicates of between 4 and 8 wells.
Control wells of GST alone and GST-NOGO-A56 were treated with HBSS without
supplements. After 2 hours pretreatment at 37C purified, dissociated cerebellar
granule neurons from post-natal day 8 rat brains were added at 20-40,000
35 neurons per well in a volume of 100µl and incubated at 37C for 24 hours.
Cultures were fixed using 4% paraformaldehyde/10% sucrose in PBS for 1 hour
then neurites were stained using a polyclonal anti-beta-III-tubulin antibody.

5 Neurite-outgrowth was quantitated using automated image capture and analysis on the Cellomics Arrayscan system.

The results are shown in figures 1 to 6.

Figure 1 shows the inhibitory effect of NOGO-A56 on neurite outgrowth compared with the control protein GST alone.

10 Figures 2 through 5 show the identification of function-blocking anti-NOGO antibodies 2A10/3, 2C4/1 and 15C3/3 together with a non-function blocking control antibody 12G3. Antibody 12G3 binds to NOGO56 but does not inhibit neurite outgrowth activity. The graphs show the average neurite length in cultures exposed to unpurified antibodies (in supernatants). The data shows the
15 blocking effect of 2A10/3, 2C4/1 and 15C3/3 of the neurite outgrowth inhibitory activity of NOGO-A56. The control is GST alone.

Figure 6 shows the blocking of the neurite outgrowth inhibitory effect of NOGO-A56 by purified 2A10/3.

20 **Example 7 – IN-1 has no blocking activity against human NOGO**

The neurite outgrowth assay as described in example 5, when carried out with the IN-1 antibody, shows that IN-1 does not block the inhibitory activity of human NOGO-A (figure 7).

25

Example 8 - Humanisation of 2A10

Humanised V_H and V_L constructs were prepared de novo by build up of
30 overlapping oligonucleotides including restriction sites for cloning into Rld and Rln mammalian expression vectors as well as a human signal sequence. Hind III and Spe I restriction sites were introduced to frame the V_H domain containing the CAMPATH-1H signal sequence for cloning into Rld containing the human $\gamma 1$ mutated constant region. Hind III and BsiW I restriction sites were introduced to
35 frame the V_L domain containing the CAMPATH-1H signal sequence for cloning into Rln containing the human kappa constant region.

- 5 CAMPATH-1H signal sequence: MGWSCIILFLVATATGVHS (SEQ.I.D.NO:82)

Heavy chain

One human germline sequence with 66% identity to the 2A10 V_H amino acid sequence was identified. The framework sequence of U84162 was selected for

- 10 humanisation:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYMHWRQAPGQGLEWMGIINPSGGSTSY
AQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGQWLVLNFDYWGQGTLVTVSS
(SEQ.I.D.NO:83).

- 15 The present invention therefore provides the use of SEQ.I.D.NO:83 (or a framework with less than 10 amino acid differences with SEQ.I.D.NO:83 e.g. less than 8 amino acids differences, preferably less than 6 amino acid differences, more preferably less than 4 amino acid differences e.g. 2 or 1 amino acid difference(s)) in the production of a humanised anti-NOGO antibody (particularly
- 20 an anti-NOGO antibody which binds to human NOGO-A and comprises the CDRs set forth in Table 2 and/or binds to the epitope set forth above)

Positions 93 and 94 were identified as potentially important residues in maintaining CDR conformation.

	Position (Kabat#)	2A10 V _H	U84162
25	93-94	EL	AR

It was noted that the EL motif at these positions are unusual.

The following humanised construct was designed:

Humanised V_H construct H1:

- 30 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWMGNINPSNGGTNY
NEKFKSRVTMTRDTSTSTVYMELSSLRSEDTAVYYCELGGYWGQGTTVTVSS
(SEQ.I.D.NO: 84).

35

5 Light chain

A human germline sequences with 66% identity to the 2A10 V_L amino acid sequence were identified. The framework sequence of CAA85593 was selected for humanisation:

Framework: CAA85593

10 DIVMTQSPLSLPVTLGQPASISCRSSQGLVYSDGDTYLNWFQQRPGQSPRRLIYKVSNRD
SGVPDRFSGGGSGTDFTLKISRVEAEDVGVYYCMQGTHWPYTFGQGTKLEIK
(SEQ.I.D.NO:85). Thus the present invention provides the use of SEQ.I.D.NO:85
(or a framework with less than 8 amino acid differences e.g. 6 amino acid
differences, preferably less than 4 amino acid differences e.g. 2 or 1 amino acid
15 difference(s)) in the production of a humanised anti-NOGO antibody which
humanised antibody binds to NOGO-A (particularly an antibody which comprises
the light chain of one or more (e.g. all) CDRs set forth in table 1 above and/or
binds to the epitope of NOGO-A set forth above) and neutralises the activity of
NOGO-A, particularly human NOGO-A.

20

The following framework residues were identified as potentially important in recovering affinity:

Position (Kabat#)	2A10 V _L	AAK94811
4	I	M
25 45	R	Q
46	R	L

A humanised V_L construct was designed:

construct	framework template	position (Kabat#)	Amino acid	
			human	mouse
30 L11	CAA85593	4	M	I
		45	R	Q
		46	R	L

35

5

Humanised V_L construct L11:

DIVITQSPLSLPVTLGQPASISCRSSKSLLYKDGKTYLNWFQQRPGQSPQLLIYLMSTRA
SGVPDRFSGGGSGTDFTLKISRVEAEDVGVYYCQQLVEYPLTFGQGTKLEIK

(SEQ.I.D.NO:86)

10 Plasmids encoding SEQ.I.D.NO:84 and 86 were transiently co-transfected into CHO cells and expressed at small scale to produce antibody H1L11. Plasmids encoding SEQ.I.D.NO:92 and 86 may also be transiently co-transfected into CHO cells and expressed at small scale to produce antibody H7L11.

15 Thus the invention provides a humanised antibody that binds to and neutralises the activity of NOGO, particularly human NOGO, more particularly human NOGO-A, which humanised antibody comprises a heavy chain variable region of SEQ.I.D.NO:84 and a light chain variable region of SEQ.I.D.NO:86. In a further aspect of the invention there is provided an antibody (particularly a fully human or
20 humanised antibody) that binds to and neutralises the activity of NOGO, particularly human NOGO, more particularly human NOGO-A, which antibody competitively inhibits the binding of the humanised antibody comprising a heavy chain variable region of SEQ.I.D.NO:85 and a light chain variable region of SEQ.I.D.NO:86, at equimolar concentration, to human NOGO-A. Preferably the
25 competing antibody inhibits at least 50% of the binding of the antibody comprising a heavy chain variable region of SEQ.I.D.NO:85 and light chain variable region of SEQ.I.D.NO:86 to human NOGO-A.

In accordance with the present invention there is provided an anti-NOGO
30 antibody which specifically binds to and neutralises the activity of human NOGO-A which antibody comprises a heavy chain of SEQ.I.D.NO:88 and a light chain of SEQ.I.D.NO:89. The present invention also concerns pharmaceutical compositions comprising said antibody and methods of treating a human patient, particularly a patient afflicted with stroke (such as Ischemic stroke) or Alzheimer's
35 disease. It will be apparent to those skilled in the art that SEQ.I.D.NO:88 and SEQ.I.D.NO:89 represent the heavy chain and light chain respectively prior to any processing (e.g. host cell mediated processing) for removal of a signal

- 5 sequence. Typically the processed form of SEQ.I.D.NO:88 will begin at position 20 and the processed form of SEQ.I.D.NO:89 will begin at position 20.

SEQ.I.D.NO:88

MGWSCIIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAP
10 GQGLEWMGNINPSNGGTNYNEKFKSRVTMTTRDTSTSTVYMELSSLRSED TAVYYCELGQG
YWGQGT LVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELAGAPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
15 PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK.

SEQ.I.D.NO:89

MGWSCIIILFLVATATGVHSDIVITQSPLSLPVT LGQPASISCRSSKSLLYKDGKTYLNWF
20 QQRPGQSPQLLIYLMSTRASGV PDRFSGGGSGTDFTLKI SRVEAEDVG VYYCQQLVEYPL
TFGQGT KLEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLN FYPREAKVQWKVDNALQS
GNSQESVTEQDSKDS TYSL SSTLTLSKADY EKHKVYACEVTHQGLSSPVTKSFNRGEC.

A polynucleotide encoding SEQ.I.D.NO:88 is set forth in SEQ.I.D.NO:90:

25 AAGCTTTACAGTTACTCAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTCT
TCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTGCAGCTGGTGCAGTCTGGGGCTG
AGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCTGCAAGGCATCTGGATACACCTTCA
CCAGCTACTGGATGCACTGGGTGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGGGAA
30 ATATTAATCCTAGCAATGGTGGTACTA ACTACAATGAGAAGTTCAAGAGCAGAGTCACCA
TGACCAGGGACACGTCCACGAGCACAGTCTACATGGAGCTGAGCAGCCTGAGATCTGAGG
ACACGGCCGTGTATTACTGTGA ACTGGGACAGGGCTACTGGGGCCAGGGAACACTAGTCA
CAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCCTCCAAGA
GCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG
35 TGACGGTGTCTGTGGA ACTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCC
TACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG
GCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGA

5 AAGTTGAGCCCAAATCTTGTGACAAACTCACACATGCCACCGTGCCCAGCACCTGAAC
TCGCGGGGGCACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGACACCCTCATGATCT
CCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCA
AGTTCAACTGGTACGTGGACGGCGTGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGG
AGCAGTACAACAGCACGTACCGTGTTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGC
10 TGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGA
AAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT
CCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATC
CCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCA
CGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACA
15 AGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACA
ACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAATTC
(SEQ. I. D. NO: 90)

A polynucleotide encoding SEQ.I.D.NO:89 is set forth as SEQ.I.D.NO:91:

20 AAGCTTTACAGTTACTCAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTCTTCTTG
GTAGCAACAGCTACAGGTGTCCACTCCGATATTGTGATAACCCAGTCTCCACTCTCCCTGCCCCGT
CACCCTTGACAGCCGGCCTCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTATATAAGGATGGGA
AGACATACTTGAATTGGTTTCAGCAGAGGCCAGGCCAATCTCCACAGCTCCTAATTTATTTGATG
25 TCCACCCGTGCATCTGGGGTCCCAGACAGATTCAGCGGCGGTGGGTGAGGCACTGATTTACACT
GAAAATCAGCAGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCCAACAACCTTGTAGAGTATC
CGCTCACGTTTGGCCAGGGGACCAAGCTGGAGATCAAACGTACGGTGGCTGCACCATCTGTCTTC
ATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAA
CTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAAGTGGACAACGCCCTCCAATCGGGTAACTCCC
30 AGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTG
AGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTC
GCCCCTCACAAAGAGCTTCAACAGGGGAGAGTGTTAGGAATTC
(SEQ. I. D. NO: 91)

35

Example 9 - BiaCore Analysis of H1L11 Anti NOGO Monoclonal Antibody

40 The binding kinetics of the anti-NOGO monoclonal antibody (mAb) to recombinantly expressed human NOGO-A (hNOGO) was analysed using the Biacore3000 biosensor. The methodology was as followed:

5 Method

hNOGO was immobilised to a CM5 chip by primary amine coupling using the Biacore Wizard program designed for targeted immobilisation levels. The CM5 sensor surface was activated by passing a solution of 50mM N-hydroxy-succinimide (NHS) and 200mM N-ethyl-N'-dimethylaminopropyl carbodi-
 10 (EDC). Then using a 300nM solution of hNOGO in 5mM sodium acetate, pH5.0, a range of concentrations between 50-200 resonance units of hNOGO were immobilised. After immobilisation was complete any still activated esters were blocked by an injection of 1M ethanolamine hydrochloride, pH8.5.

15 The H1L11 mAb (see example 8) was diluted down in HBS-EP (10mM HEPES, pH 7.4, 150mM NaCl, 3mM EDTA, and 0.005% P-20 surfactant) and binding studies were carried out at concentrations ranging between 0.1-100nM. For kinetic analysis a flowrate of 60µl/minute was used, with no mass transfer effects seen. All concentrations were performed in duplicate, in a random order
 20 with buffer blanks included. Regeneration was achieved either by the single injection of a 15µl pulse of 50mM sodium hydroxide alone or the injection of 15µl of 100mM H₃PO₄, followed by a subsequent injection of 15µl of 50mM sodium hydroxide. Both regeneration protocols were run at a flowrate of 30µl/minute; and both methods resulted in the complete removal of bound H1L11 but did not result
 25 in any loss of binding capacity of the hNOGO sensor surface. All runs were referenced against a blanked sensor surface (one that had been activated and blocked as described earlier but had no addition of ligand). Analysis of binding was carried out using the BIAevaluation kinetic analysis software version 4.1. Biacore analysis of other antibodies of the invention essentially followed the
 30 same protocol as described herein.

Results

Antibody	k_a (1/Ms)	k_d (1/s)	KD (nM)
HcLc	2.23×10^6	2.49×10^{-3}	1.26
H1L11	1.0×10^6	2.15×10^{-2}	22.16

Mouse 2A10	2.9×10^6	1.84×10^{-3}	0.8
Mouse 2C4	8.09×10^6	6.44×10^{-4}	8
Mouse 15C3	4.07×10^4	8.28×10^{-4}	17.5

5

A similar analysis was carried out using rat NOGO-A.

Results:-

Antibody	Ka (1/Ms)	Kd (1/s)	KD (nM)
Mouse 2C4	1.62×10^5	5.33×10^{-4}	3.9
Mouse 15C3	4.6×10^4	8.7×10^{-4}	24.9
Mouse 2A10	1.26×10^6	1.01×10^{-3}	1

10

Example 10 – Effect of anti-NOGO antibody (2C4) on lesion volume and functional recovery following tMCAO.

15 Aim:

The aim of this study was to investigate the efficacy of an anti-NOGO monoclonal antibody (mab) dosed intracerebroventricularly (ICV) on lesion volume and functional recovery of rats post transient middle cerebral artery occlusion (tMCAO). Cylinder test, tapered beam and neurological score tasks were utilised to assess long term functional recovery following 90 minutes of transient ischaemia and brains were harvested for immunohistochemistry evaluation of regenerative processes and lesion volume assessment.

25 Methods:

Surgical Preparation

- 5 Male Sprague-Dawley rats (300-350g) supplied by Charles River, were used in the study. Intra-cerebral ventricular (i.c.v.) cannulae were positioned in the left lateral cerebral ventricle under general anaesthesia. Following at least 4 days recovery from surgery, cannulae placement was confirmed by challenge with Angiotensin II administered ICV.

10

Induction of Focal Ischaemia

15

- All animals underwent tMCAO under halothane/oxygen/nitrous oxide anaesthesia as described by Longa and co-workers (Stroke, 1989, 20, 84-91). Body temperature was monitored throughout the surgical procedure by a rectal thermometer, and the animals were maintained normothermic ($37 \pm 0.50^\circ\text{C}$) via a heating blanket controlled by the thermometer. Actual core temperature values were recorded at the time of left middle cerebral artery (MCA) occlusion and at reperfusion.

20

- Ninety minutes after MCA occlusion, the rats were re-anaesthetised and the filament slowly and completely withdrawn to allow reperfusion. The arteriotomy is closed with diathermy, haemostasis was re-checked, and the cervical wound sutured closed.

25

Post-Occlusion Recovery

- 30 Following MCA occlusion, anaesthesia was discontinued and the animals allowed to regain consciousness and righting reflex under strict observation in an incubator ($23-25^\circ\text{C}$) for 1 hour. The animals were then housed individually in the post-operative recovery room, where their overall health status was closely monitored throughout the survival period.

35

Behavioural Tasks:

1. Neurological Assessment:

- 5 Motor and behavioural changes following MCA occlusion were assessed using a 28 point grading scale at time points 1 hour, 24 hours and then weekly for 8 weeks after MCA occlusion.

Details of Neurological assessment ("neuroscore") used:

10

Neuroscore of MCAO animals when compared to other rats within the same study – Maximum score 28 points.

- 15 Paw placement

Hold the animal lengthways at the edge of bench, cupping your hand over its head, and, one by one, take each paw and place it over the edge of the bench. Watch for paw retraction, and placement back on the bench.

20

Score: 1 for each successful paw placement (Max score=4)

Righting Reflex

- 25 Grasp the animal firmly, and rotate it until it is lying on its back in the palm of your hand. Release grip and see if the animal rights itself

Score: 1 for successful righting

- 30 Horizontal Bar

Place the fore-paws of the animal on a ribbed bar and allow it to hang.

Score: 3 if both hindlimbs rise onto the bar

2 if one hindlimb rises to bar

- 35 1 if animal just hangs

0 if animal falls off (due to lack of grip)

5

Inclined Platform

Hold cage lid at 45°. Place animal 'downhill' on the lid

Score: 3 if the animal rotates to face 'uphill' within 15 seconds

10

2 if it takes 15-30 seconds

1 if it takes longer than 30 seconds

0 if the animal falls off the lid due to weak/absent grip, or remains pointing 'downhill'

15

Contralateral Rotation

Hold the animal by the base of the tail and rotate the animal clockwise then anticlockwise. Watch for the animal's ability to swivel up contralaterally to the direction of rotation

20

Score: 1 for each side (Max score=2)

Visual Fore-paw Reaching

25

Hold the animal by the base of the tail with its head just below the level of the bench top. Approach the bench until the animal's vibrissae are almost touching it. The animal should arch and attempt to place its forepaws on the bench surface.

Score: 1 for each successful paw placement (Max score=2)

30

Circling

Place the animal on the floor and look for circling

Score: 4 for non-circling

35

3 tends to one side

2 for large circles > 50cm radius

1 for medium circles >15<50 cm radius

- 5 0 for spinning/tight circles <15cm radius

Contralateral Reflex

- 10 Hold animal by the base of the tail

Score: 0 for a reflex

1 for no reflex

- 15 Grip Strength

Let animal hold onto bars on cage with front paws only. Drag animal back by tail

Score : 2 – normal grip strength

1 – weakened grip strength

0 – no grip

20

Motility

Observe whilst on the floor for circling activity

- 25 **Score:** 3 - for normal motility
 2 – if lively but circling
 1 – if unsteady
 0 – if reluctant to move

30

General condition

Score: 3 - normal (good coat condition, alert, moving about, weight gain normal)

- 35 2 – Very good but weight gain less than normal
 1 - good

- 5 0 – fair (e.g. dirty coat, may not be grooming much, hunched posture, aggressive, weak muscle tone)

Maximum score (i.e. normal rat) =28

10

2. Cylinder test:

Animals were assessed weekly in the cylinder test of forepaw placement.

- 15 Animals were placed inside a transparent Perspex® cylinder 20cm in diameter and 30 cm tall for a 3 minute period. The numbers of left and right forepaw placements are assessed in this period. This test was conducted in red light conditions.

20 **3. Tapered beam walking:**

- For this test all the rats are trained in a red light room to cross a square tapered beam of 1m length at an angle of approx 40 degrees running uphill towards its home cage. This task becomes progressively more difficult as the rat moves up
25 the beam as the width of the beam decreases. The beam is marked so as to divide it into 3 sections for purposes of analysis, graded easy at the widest section (6 cm) through to hard at the tapered end (2 cm).

- Pre operative training - for 2 days each rat was taken from its home cage and
30 placed onto the beam at increasing distances (approx 4 times in total). When each rat could cross the beam without coaxing and with few foot faults it is classed as trained. Any poor performers were excluded from the test.

- Testing - each rat was placed on the beam 3 times per test session and the
35 latency to cross, number of foot slips, defined as a foot placement which does not achieve contact with the top surface of the beam, (hind and forelimb) was recorded manually and the average of these then taken. At weeks 5 for batch 1

- 5 and week 4 for batch two of the animals, analysis was changed to video recording of the test. Analysis from the recorded test was then carried out in order to increase sensitivity.

Test sessions for MCAO animals were prior to surgery, then post operative at
10 day 7 and weekly thereafter until sacrifice at week 8.

Dosing Regime

Antibody was administered at 1 hour, 24 hours, 1 week and 2 weeks following
15 occlusion.

Groups:

IgG1 control (5µg) (Group D)

Antibody 2C4 (5µg) (Group F)

Antibody 2C4 (15µg) (Group E)

20 All preparations were made in sterile saline.

Groups were blinded prior to administration.

To avoid bias introduced by the order of rats used, a Latin square design was employed.

Antibodies were dosed in a volume of 5µl stock (1mg/ml and 3 mg/ml) delivered
25 over 2min with the aid of an infusion pump (2.5 µl/min). Following injection, cannulae remained in place for a further 2 min.

Neuropathology and Quantification of Ischaemic Damage

8 weeks after MCA occlusion, rats were perfusion fixed with ice-cold 4%
30 paraformaldehyde. The animals were then decapitated and the brains stored *in situ* in ice-cold 4% paraformaldehyde, prior to dissection and processing for paraffin embedding and subsequent immunohistochemistry.

For brain volume analysis brains were serially sliced from the anterior pole to the
35 cerebellum for paraffin processing at 2mm intervals using a brain matrix. The brains are then paraffin processed and embedded in wax. 4 micron sections were collected that correspond to stereotactically pre-determined coronal planes from

5 anterior +3mm to posterior -7.5mm relative to bregma. The sections were mounted on to poly lysine coated slides prior to staining with Haematoxylin and Eosin. The sections were analysed for lesion volume and swelling using a computer based image analysis system (Datacell, hardware. Optimas Software). Lesioned areas are comprised of tissue that has not been stained by the
10 Haematoxylin and Eosin. Lesions are measured by tracing around the boundaries of the lesioned area and expressed as % lesioned area compared to contralateral non lesioned side of the brain.

Statistical Analysis:

15 The neuroscore and bodyweights data were analysed using a repeated measures ANOVA approach with time as the repeated measure. Lesion volume was analysed using a 1-way ANOVA and ANCOVA approach and lesion area was analysed using a repeated measures ANCOVA approach

20 The foot slips data were analysed separately for the fore and hind limbs, to look for a group effect, using a repeated measures ANOVA approach with week and beam difficulty as the repeated measures. To look for differences between fore and hind limbs, the foot slips data were also analysed using a repeated
25 measures ANOVA approach with week and leg as repeated measures, averaged across difficulty.

Three animals from group E (high dose Ab group) received one dose less than the other animals in this group (see below). Although, the analysis was carried
30 out with and without these animals and it showed there was no significant effect on the results and the animals were therefore included in the analysis, it is important to note that in the case of the cylinder test and the body weight profiles, when comparing animals dosed 3 times and animals dosed 4 times with controls, only animals dosed 4 times are significantly different to animals dosed with
35 control antibody.

- 5 It should be noted that during the course of this study, a number of rats died due to a putative infection. As a consequence, three animals in the highest dose group (group E) received one dose less than the other animals in this group.

Results:

- 10 Three animals from group E (high dose Ab group) received one dose less than the other animals in this group. The data was analysed with and without these animals and there was no significant effect on the results of the analysis unless stated in the text.
- 15 Figure 9 shows the lesion volume as a percentage of total brain volume in the control and 5µg and 15µg test groups. The antibody had no effect on lesion volume when compared to the control group.

Figure 10.

- 20 This figure shows neuroscore data represented as means \pm SEM. Due to the large range of data observed parametric analysis was deemed to be valid. There are significant differences between the 15 µg dosed group and control at weeks 1, 4, 7 and 8.
- There are significant differences between the 15 µg dosed group and control at
- 25 week 7 and 8 when analysed in this manner.

Cylinder Test

- See figures 11A, 11B, 11C, 11D. Cylinder data represented as mean \pm SEM for
- 30 A) both paws, B) left paw, C) right paw and D) right paw split into rats that received 3 doses of 15µg of anti-NOGO antibody, and those which received 4 doses of anti-NOGO antibody.
- The 15 µg dose of the antibody produced a significant increase in the use of the right paw.
- 35 When the high dose group is divided into rats receiving all four doses versus rats receiving only three doses of the antibody, no significant difference is seen between the two subgroups. However as shown in figure 11D, when the 4 dose

- 5 group is compared to the control group there exists a significant difference that is not seen when the 3 dose group is compared with control. Statistical analysis used Fisher's LSD test on repeated measures data.

10 Tapered Beam

See Figure 12.

Figure 12 shows forelimb foot slips represented as mean \pm 95% confidence intervals.

- * In week 6, group F, the 5 μ g dose is increasing the number of fore foot slips compared to group D, control, ($p=0.0305$).

Although not shown here, number of forepaw foot slips increased with increasing difficulty along the beam.

See figure 13.

- 20 Figure 13 shows hindlimb footslips represented as mean \pm 95 %confidence intervals.

- * Group F, the 5 μ g dose is significantly increasing the number of hind foot slips compared to group D, control, ($p=0.0488$) over the course of the study. In weeks 6 and 7, group F is significantly increasing the number of hind foot slips compared to group D, ($p=0.0098$ and $p=0.0370$ respectively).

Although not shown here, number of hindpaw foot slips increased with increasing difficulty along the beam.

- The data for latency for animals to cross the beam shows that in the initial week's latency decreases as the animals recover, however no effect of treatment is observed.

Figure 14 A) shows body weights represented as means \pm SEM. Dosing animals with 15 μ g of the antibody causes an increase in body weight at 24 hours, 1 week and at every time point from week 3 to the completion of the study.

- 35 Figure 14B). Graph shows weights for the 15 μ g dosed group split into animals dosed 3 times and those dosed four times. Data expressed as means \pm 95% confidence intervals. * $P < 0.05$, repeated measures ANOVA.

5 Figure 14C). Graph shows weights for the 15 µg dosed group split into animals dosed 3 times and those dosed four times. Compared to animals dosed with 5 µg of the anti-NOGO antibody and animals dosed with control antibody. Data expressed as means \pm 95% confidence intervals. * $P < 0.05$, repeated measures ANOVA.

10

Although not significantly higher than the other groups, the high dose group had a high proportion of animals removed through euthanasia or death. This could account for the mean rise in body weight when compared to the control group.

15 Conclusions:

This study looked at the effect of two doses of the anti-NOGO antibody 2C4 on lesion volume and functional recovery after a 90 minute tMCAO when administered ICV at 1 hour, 24 hours, 1 week and 2 weeks.

20

- The antibody treatment had no effect on lesion volume.
- The antibody treatment had a modest, but significant effect on neurological score at the highest dose of 15µg ICV. This dose significantly increased the neurological score at weeks 1, 4, 7 and 8 using the mean score (used as the data where spread over a large range, therefore justifying parametric analysis).

25

- The antibody treatment had a significant effect on right paw use in the cylinder test at the highest dose, 15µg ICV. This dose caused a significant increase in right paw use compared to control over the entire period of the study, there were also significant increases in right paw use specifically at weeks 2 and 6.

30

- The antibody treatment had no positive effect on performance on the tapered beam walking test. However, it appears that the low dose antibody group (5 µg ICV) increased the number of footslips, especially at week 6. However no difference was seen when the number of footslips per difficulty section were analysed, nor when latency to cross the beam was analysed.

35

- 5 • The antibody treatment had a significant effect on body weight at the highest dose, 15 µg ICV. This antibody group had a significantly increased body weight when compared to controls from 3 weeks onwards. Although not a functional outcome, body weight is a good reflection of general well being and this increase would reflect improved physiological recovery following tMCAO.
- 10

The last 3 rats in the group receiving the higher dose of the antibody did not receive the fourth and final dose for the reasons given above. In order to determine the importance of this final dose a comparison of rats in the highest antibody dose group receiving 3 doses as opposed to 4 doses was carried out.

15

Duration of Dosing

Animals dosed 4 times significantly increased number of right paw placements whereas animals dosed 3 times did not. In addition, dosing in the second week appears to accelerate the increase in body weight when compared to animals dosed 3 times. Dosing 4 times also produces a significant increase in weight compared to controls whereas dosing 3 times does not.

20

Dosing did not affect lesion volume, neurological score or performance in the tapered beam walking test

25

Summary

In summary, although the treatment had no effect on lesion volume, the highest dose of anti-NOGO antibody 2C4 (15µg) had a positive effect on functional recovery, as assessed by neuroscore set forth herein, following 3 or 4 doses and paw placement and body weight following administration of 4 doses.

30

Example 11 – Effect of Intravenously administered anti-NOGO monoclonal antibodies on lesion volume and functional recovery following tMCAO.

35

5 Aim:

The aim of this study was to investigate the efficacy of the anti-NOGO monoclonal antibodies (mabs) 2A10 and 2C4 dosed intravenously (IV) on lesion volume and functional recovery of rats post transient left middle cerebral artery occlusion (tMCAO). Cylinder test, tapered beam and neurological score tasks were utilised to assess long term functional recovery following 90 minutes of transient ischaemia and brains were harvested for immunohistochemistry evaluation of regenerative processes and lesion volume assessment.

Methods:

15

Induction of Focal Ischaemia

All animals underwent tMCAO under halothane/oxygen/nitrous oxide anaesthesia as described by Longa and co-workers (Stroke, 1989, 20, 84-91). Body temperature was monitored throughout the surgical procedure by a rectal thermometer, and the animals were maintained normothermic ($37 \pm 0.50^\circ\text{C}$) via a heating blanket controlled by the thermometer. Actual core temperature values were recorded at the time of MCA occlusion and at reperfusion. Ninety minutes after MCA occlusion, the rats were re-anaesthetised and the filament slowly and completely withdrawn to allow reperfusion. The arteriotomy is closed with diathermy, haemostasis was re-checked, and the cervical wound sutured closed.

25

Post-Occlusion Recovery

Following MCA occlusion, anaesthesia was discontinued and the animals allowed to regain consciousness under strict observation in an incubator ($23-25^\circ\text{C}$) for 1 hour. The animals were then housed individually in the post-operative recovery room, where their overall health status was closely monitored throughout the survival period.

35

Behavioural Tasks:

5 Neurological Assessment:

Motor and behavioural changes following MCA occlusion were assessed using a 28 point grading scale ("neuroscore", see example 10 for details) at time points 1 hour, 24 hours and then weekly for 8 weeks after MCA occlusion.

10

Cylinder test:

Animals were assessed weekly in the cylinder test of forepaw placement.

Animals were placed inside a transparent perspex cylinder 20cm in diameter and 30 cm tall for a 3 minute period. The number of left, right and both forepaw

15 placements whilst spontaneously rearing to explore the environment are assessed in this period. This test was conducted in red light conditions.

Tapered beam walking:

For this test all the rats are trained in a red light room to cross a square tapered
20 beam of 1m length at an angle of approx 40 degrees running uphill towards its homecage. This task becomes progressively more difficult as the rat moves up the beam as the width of the beam decreases.

25 Pre operative training - for 2 days each rat was taken from its homecage and placed onto the beam at increasing distances (approx 4 times in total). When each rat could cross the beam without coaxing and with few footfaults it is classed as trained. Any poor performers were excluded from the test.

30 Testing - each rat was placed on the beam 3 times per test session and the latency to cross, number of footslips, defined as a foot placement which does not achieve proper contact with the top surface of the beam, (hind and forelimb) was recorded by video, assessed at a later date and the average of these then taken. Test sessions for MCAO animals were prior to surgery, then post op at day 7 and weekly thereafter until sacrifice at week 8.

35

Dosing Regime (IV)

- 5 Antibody was administered intravenously at 1 hour, 24 hours, 1 week and 2 weeks following occlusion.

Groups:

A - 2C4 3mg/kg (4.7mg/kg adjusted due to low MW fractions)

B - 2A10 3mg/kg

- 10 C - Control IgG2a 3mg/kg

D - 2A10 10mg/kg

All preparations were made in sterile saline.

Groups were blinded prior to administration.

- To avoid bias introduced by the order of rats used, a Latin square design was
15 employed. Animals were randomised and operators were blinded to treatment.

Neuropathology and Quantification of Ischaemic Damage

- 8 weeks after MCA occlusion, rats were perfusion fixed with ice-cold 4%
20 paraformaldehyde. The animals were then decapitated and the brains stored *in situ* in ice-cold 4% paraformaldehyde for 48 hrs, prior to dissection and processing for paraffin embedding and subsequent histological analysis.

- For brain volume analysis brains were serially sliced from the anterior pole to the
25 cerebellum for paraffin processing at 2mm intervals using a brain matrix. The brains are then paraffin processed and embedded in wax. 4 micron sections were collected that correspond to stereotactically pre-determined coronal planes from anterior +3mm to posterior -7.5mm relative to bregma. The sections were mounted on to poly lysine coated slides prior to staining with Haematoxylin and
30 Eosin. The sections were analysed for lesion volume using a computer based image analysis system (Datacell, hardware. Optimas Software). Lesioned areas are delineated by areas of tissue with reduced Haematoxylin and Eosin. Lesions are measured by tracing around the boundaries of the lesioned area and expressed as % lesioned area compared to contralateral non lesioned side of the
35 brain.

Statistical Analysis

5

The neuroscore and bodyweights data were analysed using a repeated measures ANOVA approach with time as the repeated measure. Lesion volume was analysed using a 1-way ANOVA and ANCOVA approach and lesion area was analysed using a repeated measures ANCOVA approach

10

The footslips data were analysed separately for the fore and hind limbs, to look for a group effect, using a repeated measures ANOVA approach with week and beam difficulty as the repeated measures. To look for differences between fore and hind limbs, the footslips data were also analysed using a repeated measures ANOVA approach with week and leg as repeated measures, averaged across difficulty.

15

Results:

20 Lesion Volume:

Figure 15 represents the lesion volume as a percentage of total brain volume. The antibody had no effect on lesion volume when compared to the control group.

25 Neuroscores

Figure 16 shows the neuroscore data represented as means \pm SEM. Due to the large range of data observed parametric analysis was deemed by to be valid.

* $P < 0.05$ compared to control antibody, repeated measures ANOVA.

30

Cylinder Test

See Figures 17A, 17B and 17C. Cylinder data represented as mean \pm SEM for A) both paws, B) left paw, C) right paw. * $P < 0.05$, ** $P < 0.01$ versus control antibody, repeated measures ANOVA.

35

Tapered Beam

5

Forelimb footslips:

See Figure 18. Forelimb footslips represented as mean \pm SEM. *P< 0.05, **P<0.01 versus control antibody, repeated measures ANOVA.

10

Hind Limb footslips

See Figure 19. Hindlimb footslips represented as mean \pm SEM. *P< 0.05 versus control antibody, repeated measures ANOVA.

15

Latency to cross the beam

See Figure 20. Hindlimb footslips represented as mean \pm SEM. *P< 0.05 versus control antibody, repeated measures ANOVA.

20

Conclusions:

This study looked at the effect of two doses of the anti-NOGO antibody 2A10 (3 & 10 mg/kg) and one dose of 2C4 (3 mg/kg) on lesion volume and functional recovery after a 90 minute tMCAO when administered IV at 1hour, 24 hours, 1 week and 2 weeks.

25

- The antibody treatment had no effect on lesion volume.
- The antibody treatment had a significant effect on neurological score at the highest dose of 2A10 (10 mg/kg) at 24 hours.
- The 2A10 antibody treatment (3mg/kg) had a significant effect on right paw use in the cylinder test specifically at weeks 1 and 2.
- 2A10 (3mg/kg) had a clear effect of reducing the number of fore paw footslips at 1 and 2 weeks following tMCAO, 2A10 (10mg/kg) had the effect of reducing hind paw footslips at 2 weeks following tMCAO, and both concentrations of 2A10 reduced latency to cross the beam at 1 week following tMCAO. 2C4 (3 mg/kg) reduced forepaw footslips at 3 weeks following tMCAO relative to control antibody.

30

35

5

Summary

10 In summary, although the treatment had no effect on lesion volume, intravenous injection of both the anti-NOGO monoclonal antibodies 2A10 and 2C4 had a positive effect on functional recovery, as assessed by neurological score, paw placement and tapered beam predominantly in the first two weeks following tMCAO.

15 **Example 12 – Transfection of NOGO cDNA into SHSY5Y-APP cells**

The day before transfection the SHSY5Y-APPwt cells were trypsinised, counted and replated at 200,000 to 1 million cells per well of a 6-well plate (Nunc). The NOGO expression construct (FLAG-tagged NOGO-A cDNA in pCDNA3 (Invitrogen); MYC-tagged NOGO-B and NOGO-C in pCDNA3.1A) was
20 complexed with PlusTM reagent by diluting the DNA into serum free medium (OptiMEM-1), adding PlusTM reagent, mixing and incubating at room temperature for 15 min. (6µl Plus reagent into a total volume of 100µl with 2µg DNA and OptiMEM-1 per well)

25 LipofectAMINETM reagent was diluted into serum free medium (Optimem-I) in a second tube and mixed (4µl Lipofectamine in 100µl volume per well). Pre-complexed DNA (from above) was combined with the diluted lipofectAMINE, mixed and incubated at room temp for 15 min.

Meanwhile the cells were washed with serum free medium (OptiMEM-I) and then
30 fresh serum free medium was added to the cells (800µl per well). The DNA-Plus-LipofectAMINE reagent complexes were then added to the cells (200µl), mixed gently and the cells incubated at 37 °C for 5hr in 5% CO₂. After 5 hours 1ml serum containing growth medium was added to the cells and the cells incubated overnight or 2 hours.

35 After 14 hours (or 2 hours) all medium was removed and replaced with 1ml (OptiMEM-1) per well.

- 5 After 48 hours conditioning the medium was collected and assayed for amyloid content as described in example 13.

10

Example 13 – Detection of A β peptide by IGEN ELISA

- 15 SHSY5Y cells overexpressing the human APPwt or Amyloid Precursor Protein Swedish variant sequence (APPswe) were seeded in 6 well or 96 well Nunc plates at the required density.
- After 24 hours the reagents (eg. antibody, peptides, compounds etc) for testing were added to the cells in a final volume of 120 μ l and cells incubated for 24hr.
- 20 The medium was removed from cells and 50 μ l was assayed for A β x-40 and 50 μ l for A β x-42 in an overnight ORIGEN immunoassay employing A β C-terminal specific antibodies. Briefly, A β peptides were captured using biotinylated 6E10 (Signet Labs). Ori-tagged labelled A β C-terminal specific antibodies were used to detect the A β x-40 and A β x-42 species. Antibody- A β complexes were captured
- 25 with streptavidin coated dynabeads and assayed in an IGEN M8 analyser. The viability of the cells was checked using MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Thiazolyl blue) reagent. Briefly, MTT reagent (5mg/ml in phosphate buffered saline) was diluted 1:10 in culture medium and 100 μ l added to each well. Following incubation at 37°C for 4hr, 100 μ l solubilising
- 30 solution (20% SDS/50% Dimethyl Formamide) was added. Absorbance of plates was read using a Delfia Wallac plate reader at 590nm.

The results are shown in figures 21 to 34.

- 35 SHSY5Y-APPwt cells express wild-type APP. SHSY5Y-APPswe cells express the Swedish mutation form of APP.

5

Example 14 - The effect of NOGO-A expression on levels of secreted A β 40 and A β 42 peptide

When an expression construct expressing NOGO-A is introduced into either
10 SHSY5Y-APPwt cells or SHSY5Y-APPswe cells the levels of A β 40 and A β 42 are seen to significantly increase suggesting that NOGO-A is in some way modulating, directly or indirectly, the proteolytic processing of APP and/or degradation of A β peptides. The fact that the product of this altered APP processing is the peptide A β 40 could suggest that the effect of NOGO could be
15 at the level of modulating β -secretase activity.

Figure 21 shows the increase in the level of secreted A β 40 when NOGO-A is expressed from an expression vector. The two left-hand bars are the controls (vector alone and vector carrying a control protein, green fluorescent protein (GFP)), showing the background levels of A β 40 production in this cell-line. The
20 remaining bars shows that significantly increased level of A β 40 peptide are detected when NOGO-A, fused to a FLAG peptide, is expressed in the cells and also that a similar elevation, albeit less marked, when NOGO-B, fused to myc, is expressed.

The same experiment was repeated using an ELISA specific for A β 42. The
25 results showed a similar elevation in levels of A β 42 peptide secretion as that seen in the earlier experiment using A β 40 ELISA. Again NOGO-B also showed increased secretion of A β 42 peptide and again the increase was less marked than with NOGO-A. The results are shown in figure 22.

Repeat experiments comparing levels of secreted peptide from cells transfected
30 with NOGO-A compared to vector pCDNA3 alone, are shown in figures 23 (for A β 40) and 24 (for A β 42).

A direct comparison of levels of A β 40 and A β 42 peptide secreted is shown in figure 25. Figure 25 is the average of three to five separate experiments to confirm the consistency of NOGO.

35

- 5 Thus the invention provides the use of an anti-NOGO antibody (particularly an anti-NOGO-A and/or anti-NOGO-B antibody) in the manufacture of a medicament for the treatment of Alzheimer's disease.

Example 15 - anti-NOGO-A antibody inhibits A β 40 and A β 42 peptide

10 **secretion from SHSY5Y-APPwt and SHSY5Yswe cells**

Figure 26 shows the dramatic reduction in levels of A β 40 and A β 42 peptide secreted from SHSY5Y-APPwt cells expressing endogenous NOGO-A when the anti-NOGO antibody 2A10 is introduced into the culture medium.

- 15 The effect is seen in a dose-dependent manner, at 30 μ g/ml reaching inhibition levels of almost 90%. There is no apparent difference in the effect between A β 40 and A β 42 peptides (white bars and black bars respectively in figure 26), in other words any effect of the anti-NOGO antibody on APP processing is not preferential for either the A β 40 or A β 42 peptides.

- 20 Figure 27 shows the same experiment as in figure 26 but with an unrelated IgG1 antibody. As can clearly be seen in the figure, an unrelated (non anti-NOGO A) monoclonal antibody has no inhibitory effect on the levels of A β 40 and A β 42 peptide secretion from the cells.

- Figure 28 shows that the same unrelated IgG1 antibody similarly shows little or
25 no inhibitory effect on the levels of A β 40 and A β 42 peptide secretion from SHSY5Y-APPswe cells expressing NOGO-A.

- Similarly figure 29 shows the results of the same experiment as described above for figure 26 but using an anti-NOGO monoclonal antibody which binds to NOGO-A but is not a function-blocker (6D5). The non-function-blocking anti-
30 NOGO-A monoclonal antibody has minimal effect (less than 10%) on the secretion of A β 40 and A β 42 peptides from SHSY5Y-APPwt cells expressing NOGO-A. This result suggests that the results shown in figure 26 are a result of the inhibition of NOGO functional activity by the anti-NOGO antibody.

- Figure 30 shows the results of the same experiment as for figure 29, using the
35 non-function blocking anti-NOGO monoclonal antibody (6D5) but with SHSY5Y-APPswe cells expressing endogenous NOGO-A. As before (figure 29) there is

5 minimal effect on the levels of A β 40 and A β 42 being secreted by this cell line, being less than 10% inhibition.

Figure 31 shows the results of an experiment which extends the results of the experiment of figure 26. Figure 31 shows that the concentration-dependent effect of the inhibitory effect shown by the function-blocking antibody 2A10/3
10 continues at a higher concentration, a level of greater than 90% inhibition being achieved at an antibody concentration of 50 μ g/ml.

Figure 32 shows the results of an identical experiment to that of figure 31 except that SHSY5Yswe cells are used. The concentration-dependent inhibitory effect of the antibody 2A10/3 continues to be seen at the higher concentration of
15 50 μ g/ml.

Figure 33 shows the effect of a different function-blocking anti-NOGO-A antibody, 2C4, on the secretion of A β 40 and A β 42 peptides from SHSY5Y-APPwt cells. The results show a concentration-dependent inhibitory effect on the levels of A β 40 and A β 42 peptide secretion to a level of 36% at a concentration of 20 μ g/ml.

20 Again the effect is seen on both A β 40 and A β 42 peptides.

Figure 34 compares the inhibitory effect of the anti-NOGO-A function-blocking antibodies 2A10, 2C4 and 15C3 (at the concentrations shown in the figure) with other control antibodies 10A4, IgG2b and 14D12. The figure shows that the effect on inhibition of A β 40 and A β 42 secretion is specific to the function-
25 blocking anti-NOGO-A monoclonal antibodies.

Example 16 – Increased NogoA expression elevates A β levels in a dose-dependent manner

30 To investigate the effect of NogoA expression on A β levels, SHSY5Y-APPwt cells were transiently transfected with increasing amounts of C-terminal myc tagged NogoA cDNA. The total amount of cDNA (5 μ g) was kept constant for each transfection using pcDNA3.1myc cDNA. 48hr post-transfection, culture medium was removed and assayed for A β 40. In addition, cells were harvested and lysed
35 in 10mM Tris/HCl containing 1% Triton X-100 and Complete protease inhibitors (Roche). Cell lysates were resolved on 10% Novex Tris-glycine gels and subject

5 to Western blot analysis with an anti-NogoA antibody. An increase in NogoA protein expression was observed with increasing NogoA cDNA concentrations. This was concomitant with a corresponding increase in A β 40 levels in the medium from these cells. Thus, increased expression of NogoA causes a dose-dependent increase in A β 40 levels. See Figure 36.

10

Example 17 – Chimeric 2A10 (HcLc)

A chimaeric antibody consisting of parent murine V regions grafted onto human
15 IgG1/k wild type C regions was designed to be used as a reference when testing humanised constructs. The NOGO 2A10 V_H domain in the mouse recombinant Rld plasmid was cut Hind III-Spe I and ligated into the Rld vector containing hCy1wt. The NOGO 2A10 V_L domain in the mouse recombinant Rln plasmid was cut Hind III-BsiW I and ligated into the Rln vector containing hCk.

20

SEQ.I.D.NO:92 sets forth the heavy chain sequence for HcLc

MGWSCIIILFLVAAATGVHSQVQLQQPGTELVKPGASVKLSCKASGYTFTSYWMHWVKQRP
GQGLEWIGNINPSNGGTNYNEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCELGQG
YWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
25 GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFF
LYSKLTVDKSRWQQGNVVFSCVMHEALHNHYTQKSLSLSPGK (SEQ.I.D.NO:92)

30

A polynucleotide encoding SEQ.I.D.NO:92 is set forth as SEQ.I.D.NO:93.

AAGCTTGCCACCATGGGATGGAGCTGTATCATCCTCTTTTGGTAGCAGCAGCTACAGGT
GTCCACTCCCAGGTCCAAGTGCAGCAGCCTGGGACTGAAGTGGTGAAGCCTGGGGCTTCA
35 GTGAAGCTGTCCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTGGATGCACTGGGTG
AAGCAGAGGCCTGGACAAGGCTTGAGTGGATTTGGAAATATTAATCCTAGCAATGGTGGT
ACTAACTACAATGAGAAGTTCAAGAGCAAGGCCACACTGACTGTAGACAAATCCTCCAGC
ACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTATTGTGAA
CTGGGACAGGGCTACTGGGGCCAAGGCACACTAGTCACAGTCTCCTCAGCCTCCACCAAG

5 GGGCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCC
 CTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGC
 GCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTTACAGTCCTCAGGACTCTACTCC
 CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
 GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGAC
 10 AAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTC
 CTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGC
 GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC
 GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGT
 GTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
 15 AAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGG
 CAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAAC
 CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGG
 GAGAGCAATGGGCAGCCGAGAACTACAAGACCACGCCTCCCGTGCTGGACTCCGAC
 GGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAC
 20 GTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC
 TCCCTGTCTCCGGGTAAATGAATTC (SEQ. I. D. NO: 93) .

The amino acid sequence of the light chain of HcLc is set forth as

25 SEQ. I. D. NO: 94:

MRCSLQFLGVLMFWISGVSGDIVITQDELSNPVTSGESVSI SCRSSKSLLYKD GKTYLNW
 FLQRPQGSPQLLIYLMSTRASGVSDRFSGSGSGTDFTLEISR VKAEDVG VYYCQQLVEYP
 LTFGAGTKLELKR TVAPS VFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQ
 30 SGNSQESVTEQDSKDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
 (SEQ. I. D. NO: 94)

A polynucleotide encoding SEQ. I. D. NO: 94 is set forth as SEQ. I. D. NO: 95 :

35 AAGCTTGCCACCATGAGGTGCTCTCTTCAGTTTCTGGGGGTGCTTATGTTCTGGATCTCT
 GGAGTCAGTGGGGATATTGTGATAACCCAGGATGAACTCTCCAATCCTGTCACTTCTGGA
 GAATCAGTTTCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTATATAAGGATGGGAAGACA
 TACTTGAATTGGTTTCTGCAGAGACCAGGACAATCTCCTCAGCTCCTGATCTATTTGATG
 TCCACCCGTGCATCAGGAGTCTCAGACCGGTTTAGTGGCAGTGGGTCAGGAACAGATTTC
 40 ACCCTGGAAATCAGTAGAGTGAAGGCTGAGGATGTGGGTGTGTATTACTGTCAACAACTT
 GTAGAGTATCCGCTCACGTTCCGGTGCTGGGACCAAGCTGGAGCTGAAACGTACGGTGGCT

5 GCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCT
 GTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGAC
 AACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGC
 ACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTC
 TACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGG
 10 GGAGAGTGTTAGGAATTC (SEQ. I. D. NO: 95)

Example 18 – Humanised anti-NOGO antibodies bind to human NOGO

GST-human NOGO-A56 at 1µg/ml in 50mM Tris pH9.5 was coated onto Nunc
 Immunosorp plates (100µl per well) at 4 °C overnight. Wells were rinsed once
 15 with TBS + 0.05% tween then incubated with 2% BSA in TBS + 0.05% tween to
 block non-specific binding sites at room temperature for 1hour. Antibodies were
 diluted in TBS + 0.05% tween + 2% BSA to 10µg/ml and 1/2 dilutions made from
 this. Antibodies were added to wells in duplicate and incubated at at room
 temperature for 1hour. Wells were washed three times with TBS + 0.05% tween
 20 then incubated with anti-human kappa peroxidase conjugate (1:2000) for 1hour.
 Washed three times with TBS + 0.05% tween and then incubated with 100µl
 OPD peroxidase substrate (Sigma) per well for 10 minutes. The colour reaction
 was stopped by the addition of 25µl concentrated H₂SO₄. Optical density at
 490nm was measured using a plate reader. Background values read from wells
 25 with no antibody were subtracted.

Figures 35A to C illustrates the dose-dependent binding of humanised antibody
 H1L11 in comparison with the chimera (HcLc) to human NOGO-A56 in an ELISA
 assay. The Y-axis shows the measured optical density (OD) at 490nm, a
 quantitative measure of antibody captured in the wells. The X-axis shows the
 30 concentration of antibody used (ug/ml) per well at each data point. Antibodies
 H1L11 and HcLc give EC50 values of of 0.118 µg/ml and 0.022 µg/ml
 respectively.

5

CLAIMS

1. An antibody or functional fragment thereof which binds with (e.g. to) and neutralises human NOGO.

10 2. An antibody according to claim 1 which binds to a region of human NOGO-A protein between amino acids 586 to 785.

3. An antibody according to claim 2 which binds to a region of human NOGO-A between amino acids 586 to 685.

15

4. An antibody according to claim 2 which binds to a region of human NOGO-A between amino acids 686 to 785.

20 5. An antibody according to claim 1 which comprises each of the following CDRs:
Light chain CDRs: SEQ.I.D.NO:1, 2 and 3

Heavy chain CDRs:SEQ.I.D.NO: 4,5 and 6.

25

6. An antibody according to claim 1 which comprises each of the following CDRs:
Light chain CDRs: SEQ.I.D.NO:7, 8 and 9

30

Heavy chain CDRs: SEQ.I.D.NO:10,11 AND 12.

35 7. An antibody according to claim 1 which comprises each of the following CDRs:
Light chain CDRs:SEQ.I.D.NO:13,14 AND 15;

5

Heavy chain CDRs: SEQ.I.D.NO:16,17 AND 18.

- 10 8. An antibody according to claim 5 which comprises a heavy chain variable domain which comprises each of the CDRs selected from CDRH1, CDRH2 and CDRH3 and a light chain variable domain which comprises one or more CDRs selected from CDRL1, CDRL2 and CDRL3.
- 15 9. An antibody according to claim 6 which comprises a heavy chain variable domain which comprises each of the CDRs selected from CDRH1, CDRH2 and CDRH3 and a light chain variable domain which comprises one or more CDRs selected from CDRL1, CDRL2 and CDRL3.
- 20 10. An antibody according to claim 7 which comprises a heavy chain variable domain which comprises each of the CDRs selected from CDRH1, CDRH2 and CDRH3 and a light chain variable domain which comprises one or more CDRs selected from CDRL1, CDRL2 and CDRL3.
- 25 11. An antibody of any one of claims 1 to 10 which is a monoclonal antibody.
12. An antibody of any one of claims 1 to 13 which is a humanised or chimeric antibody.
- 30 13. An antibody according to claim 8 wherein the heavy chain variable region comprises the amino acid sequence set forth in SEQ.I.D.NO:37
14. An antibody according to claim 9 wherein the heavy chain variable region
- 35 comprises the amino acid sequence set forth in SEQ.I.D.NO:38.

5 15. An antibody according to claim 10 wherein the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO:39.

16. An antibody according to claim 8 or 13 wherein the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO:40.
10

17. An antibody according to claim 9 or 14 wherein the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO:41.

15 18. An antibody according to claim 10 or 15 wherein the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO:42.

20 19. A pharmaceutical composition comprising an anti-NOGO antibody or functional fragment thereof according to any preceding claim together with a pharmaceutically acceptable diluent or carrier.

20. A method of treatment or prophylaxis of stroke and other neurological
25 diseases/disorders in a human which comprises administering to said human in need thereof an effective amount of an anti-NOGO antibody, according to any one of claims 1-18 including altered antibodies or a functional fragment thereof.

21. The use of an anti-NOGO antibody according to any one of claims 1-18,
30 including altered antibodies or a functional fragment thereof in the preparation of a medicament for treatment or prophylaxis of stroke and other neurological diseases/disorders.

22. A method of inhibiting neurodegeneration and/or promoting functional
35 recovery in a human patient suffering, or at risk of developing, a stroke or other neurological disease/disorder which comprises administering to said human in

5 need thereof an effective amount of an anti-NOGO antibody according to any one of claims 1-18, including altered antibodies or a functional fragment thereof.

23. The use of an anti-NOGO antibody according to any one of claims 1-18, including altered antibodies or a functional fragment thereof in the preparation of
10 a medicament for inhibiting neurodegeneration and/or promoting functional recovery in a human patient afflicted with, or at risk of developing, a stroke and other neurological disease/disorder.

24. A method of treating or prophylaxis of stroke or other neurological
15 disease/disorder in a human comprising the step of parenteral administration of a therapeutically effective amount of an anti-NOGO antibody according to any one of claims 1 to 18 to said human.

25. The method of claim 24 wherein the anti-NOGO antibody is administered
20 intravenously.

26. The method of any one of claims 20 to 24 wherein the other neurological disease/disorder is selected from the group consisting of;
traumatic brain injury, spinal cord injury, Alzheimer's disease, fronto-
25 temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease and multiple sclerosis.

27. A method of promoting axonal sprouting comprising the step of contacting a human axon with an anti-NOGO antibody of claims 1 to 18.

30

28. The method of claim 27 wherein the method is in vitro.

29. A method of producing an anti-NOGO antibody of any one of claims 1 to 18 which specifically binds to and neutralises the activity of human NOGO-A which
35 method comprises the steps of;

- (a) providing a first vector encoding a heavy chain of the antibody;
- (b) providing a second vector encoding the light chain of the antibody;

- 5 (c) co-transfecting a mammalian host cell with said first and second vectors;
- (d) culturing the host cell of step (c) in culture media (preferably serum free) under conditions permissive to the secretion of the antibody from said host cell into said culture media;
- 10 (e) recovering the secreted antibody of step (d).

30. A method of producing an anti-NOGO antibody that competitively inhibits the binding of the antibody of any one of claims 1 to 18 which method comprises the steps of;

- 15 (a) providing a first vector encoding a heavy chain of the antibody;
- (b) providing a second vector encoding the light chain of the antibody;
- (c) co-transfecting a mammalian host cell with said first and second vectors;
- (d) culturing the host cell of step (c) in culture media (preferably serum free) under conditions permissive to the secretion of the antibody from said host cell into said culture media;
- 20 (e) recovering the secreted antibody of step (d).

31. A method of producing an intravenously administrable pharmaceutical composition comprising an anti-NOGO antibody which binds to and neutralises the activity of NOGO-A which method comprises the steps of;

- 25 (a) providing a first vector encoding a heavy chain of the antibody;
- (b) providing a second vector encoding the light chain of the antibody;
- (c) introducing (e.g.co-transfecting) said first and second vectors into a mammalian host cell;
- 30 (d) culturing the host cell of step (c) in culture media (preferably serum free) wherein said host cell secretes into said culture media an antibody comprising a light and heavy chain;
- (e) recovering (and optionally purifying) the secreted antibody of step (d);
- 35 (f) incorporating the antibody of step (e) into a intravenously administrable pharmaceutical composition.

- 5 32. A method of producing an anti-NOGO antibody which binds to human
NOGO-A between amino acids 586-785, particularly 586-685 or 686 to 785 and
neutralises the activity of said NOGO-A which method comprises the steps of;
- 10 (a) providing a first vector encoding a heavy chain of the antibody;
(b) providing a second vector encoding the light chain of the antibody;
(c) introducing (e.g.co-transfecting) said first and second vectors into a
mammalian host cell;
(d) culturing the host cell of step (c) in culture media (preferably serum
free) wherein said host cell secretes into said culture media an
antibody comprising a light and heavy chain;
- 15 (e) recovering (and optionally purifying) the secreted antibody of step (d);

33. A method according to claim 29 to 32 wherein the host cell is selected from
the group consisting of; NS0 Sp2/o, CHO, COS, a fibroblast cell such as 3T3,
particularly CHO.

20

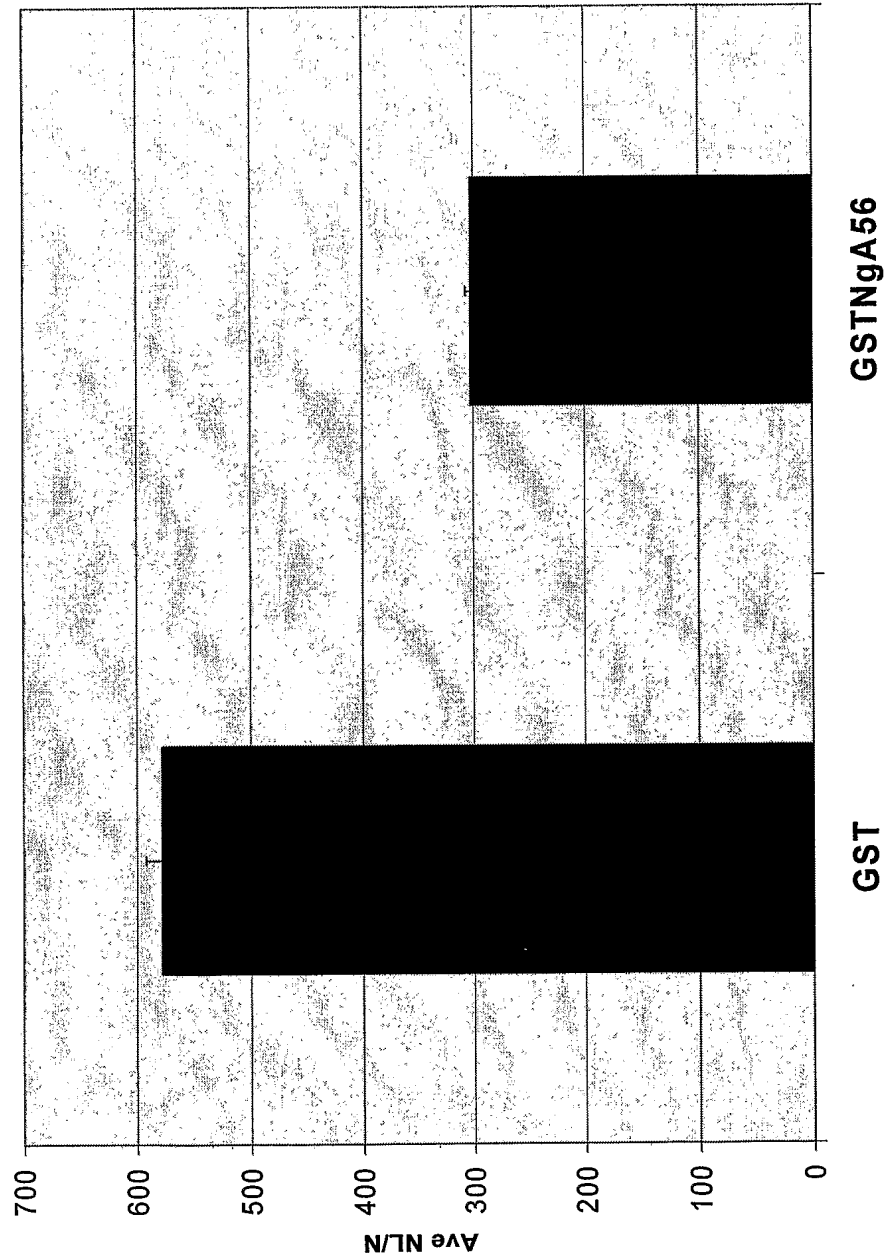


Figure 1

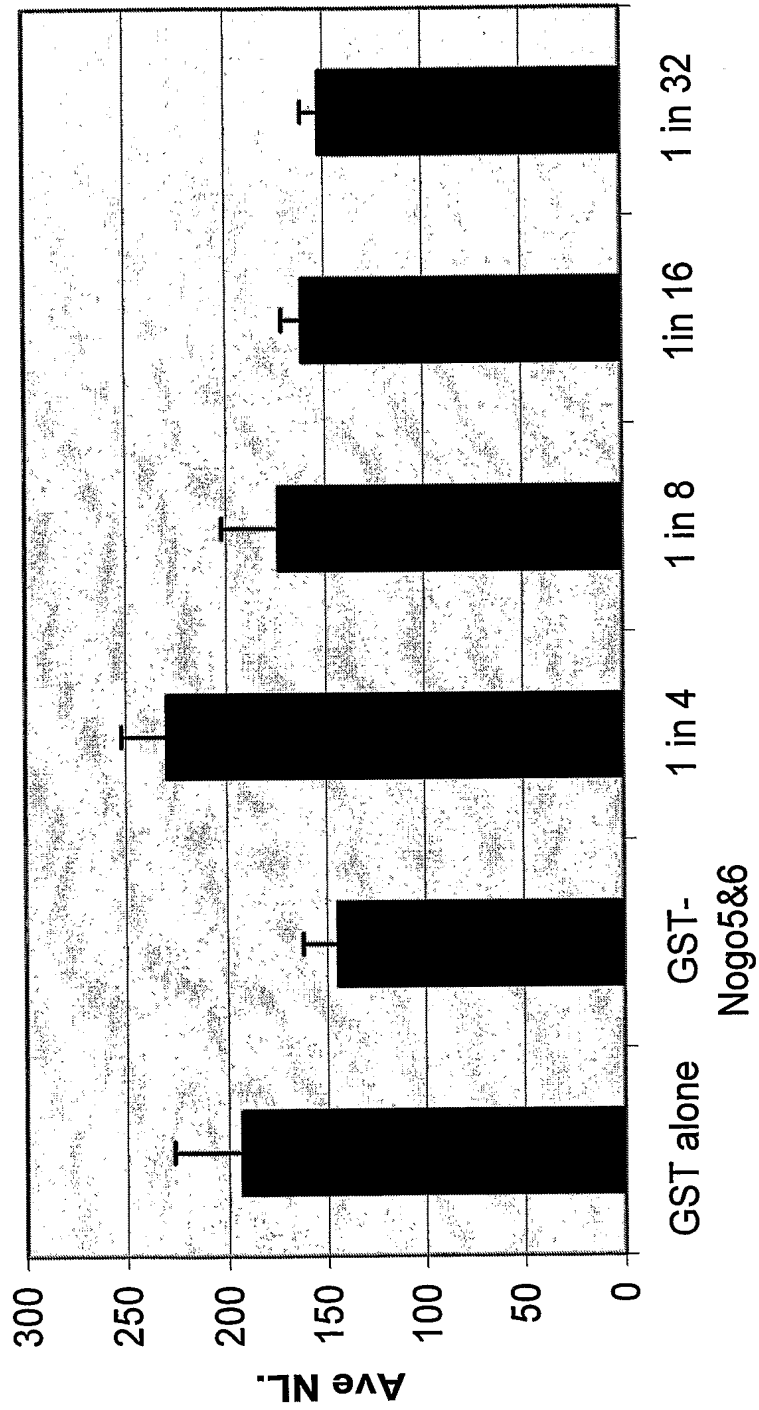
Figure 2

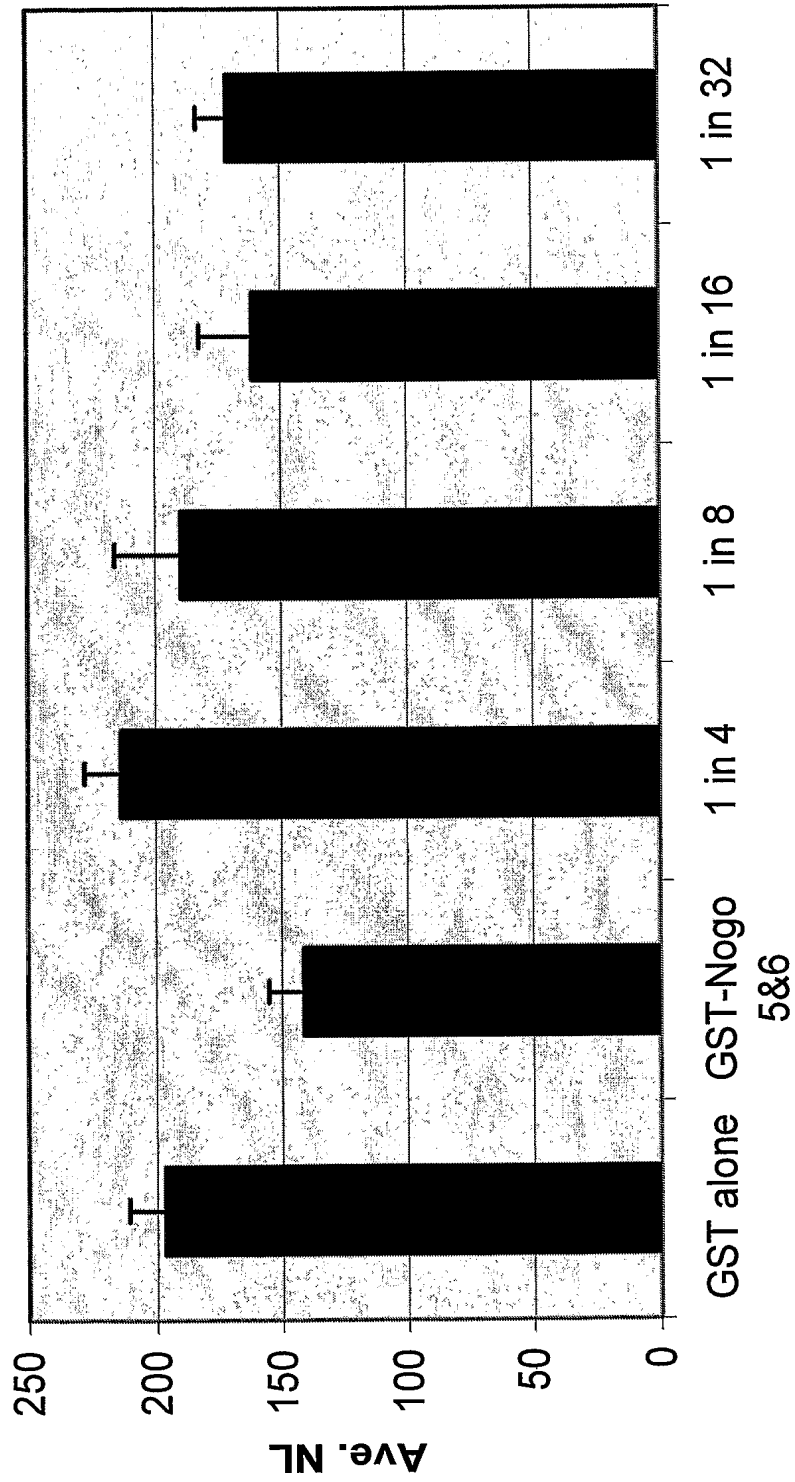
Figure 3

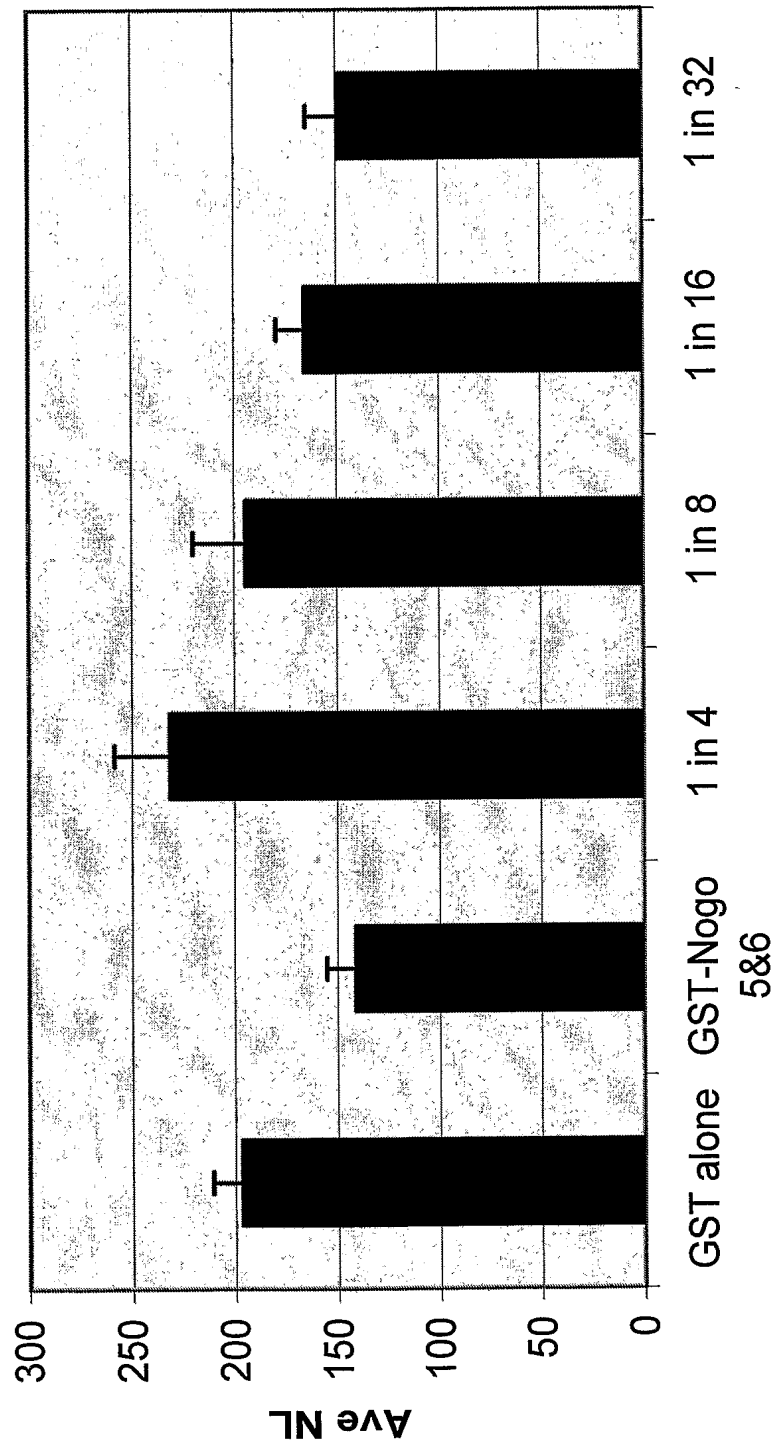
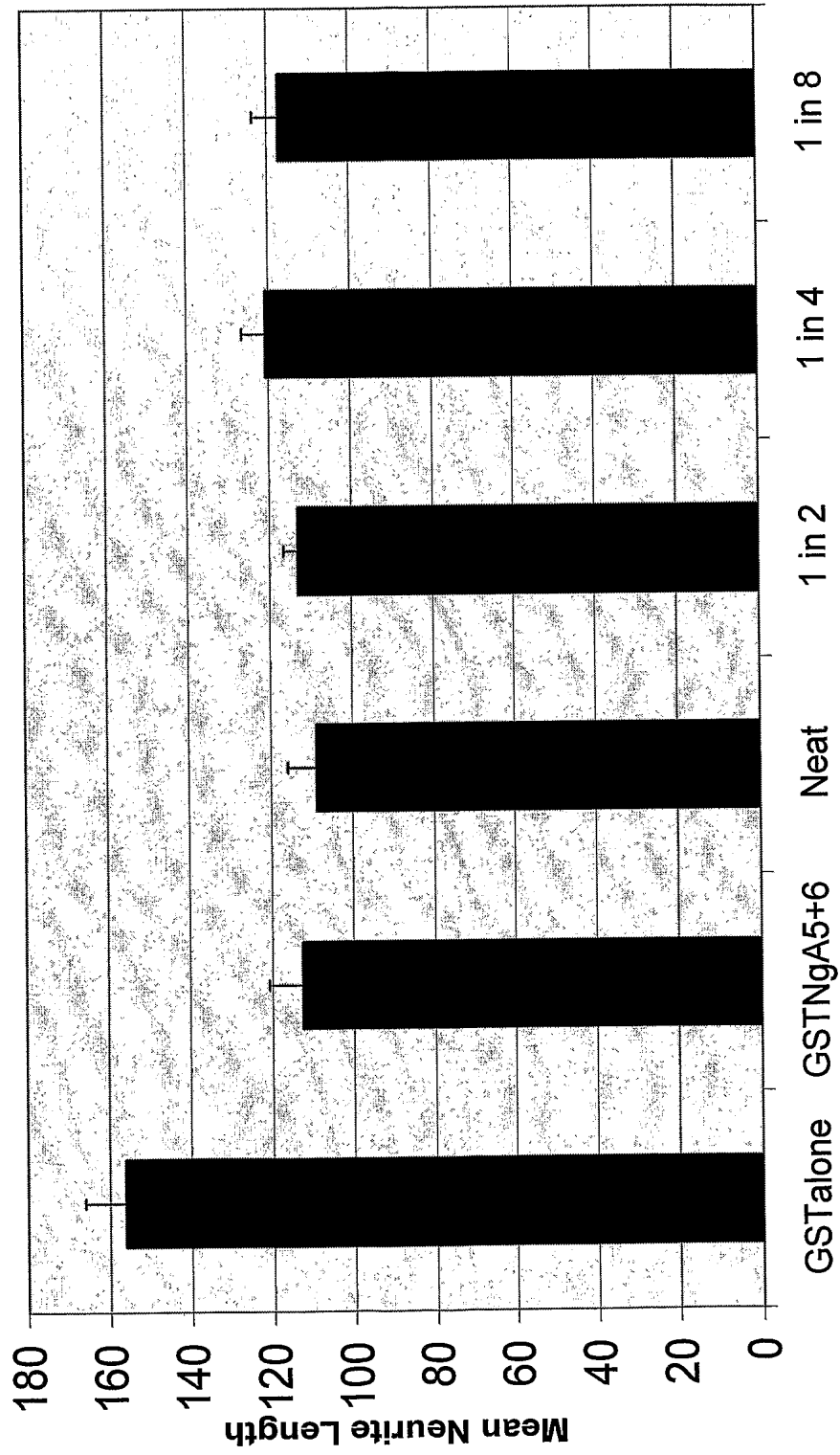
Figure 4

Figure 5

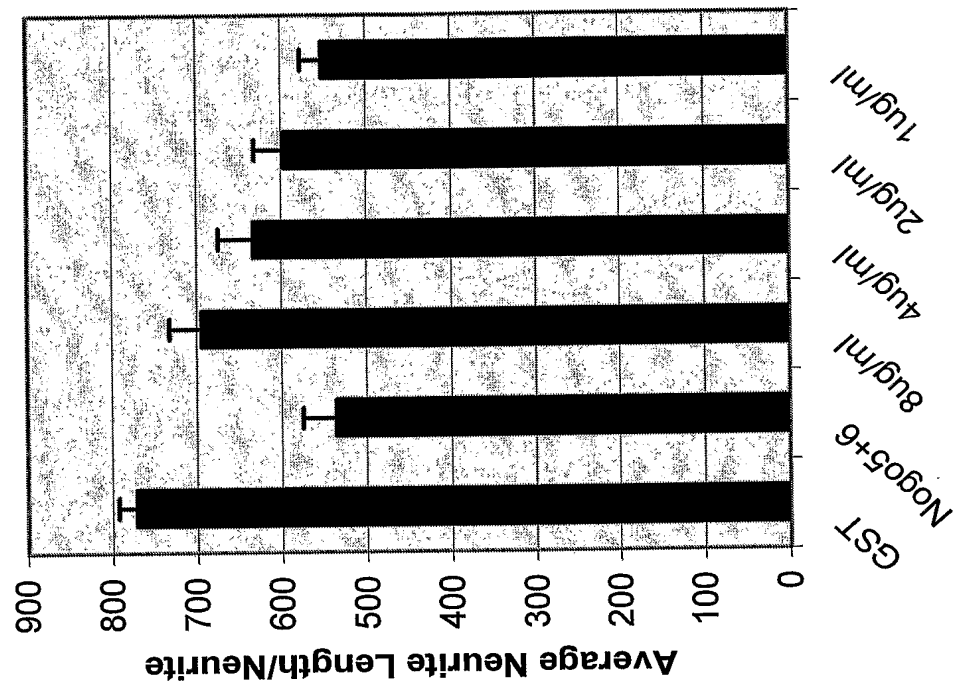
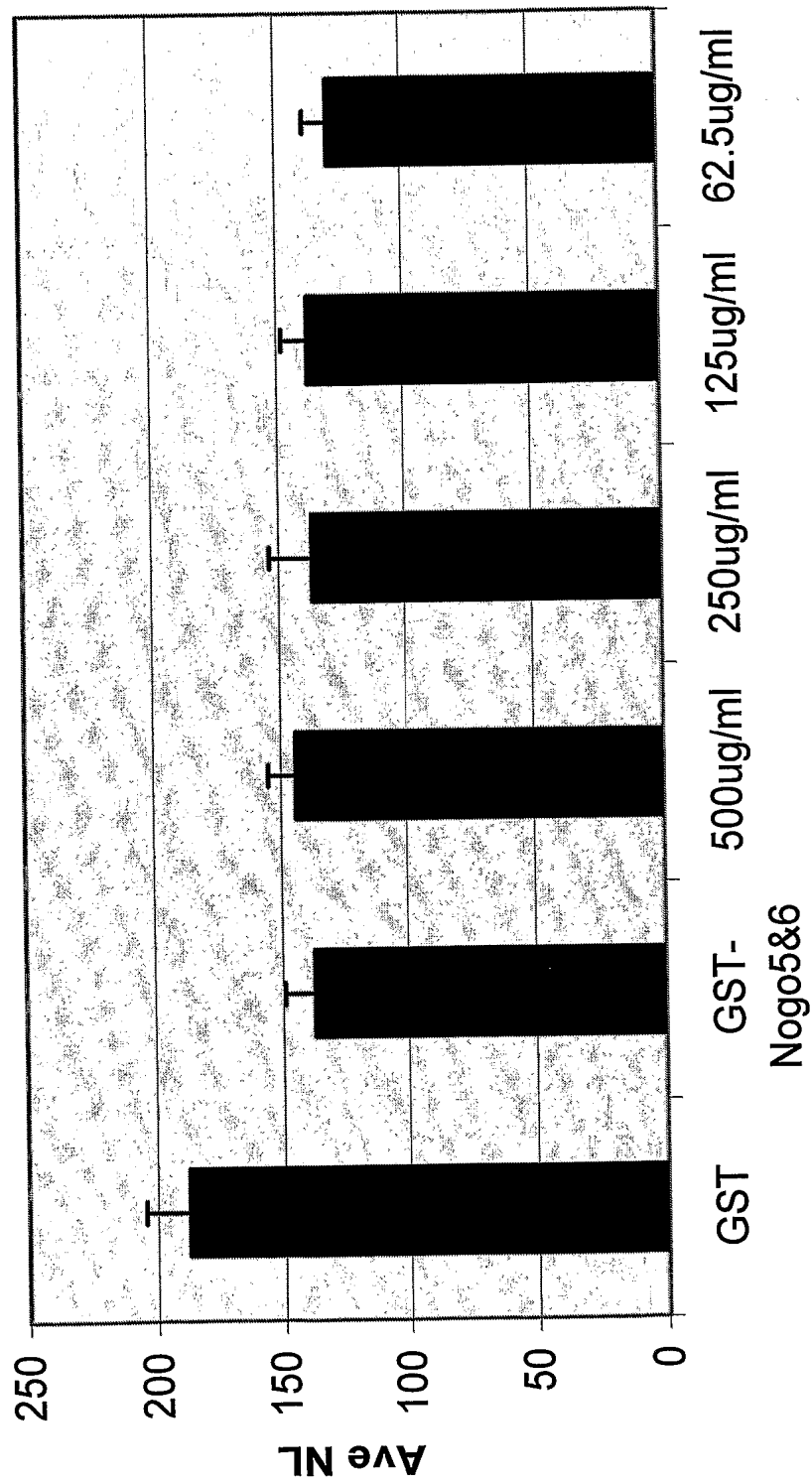
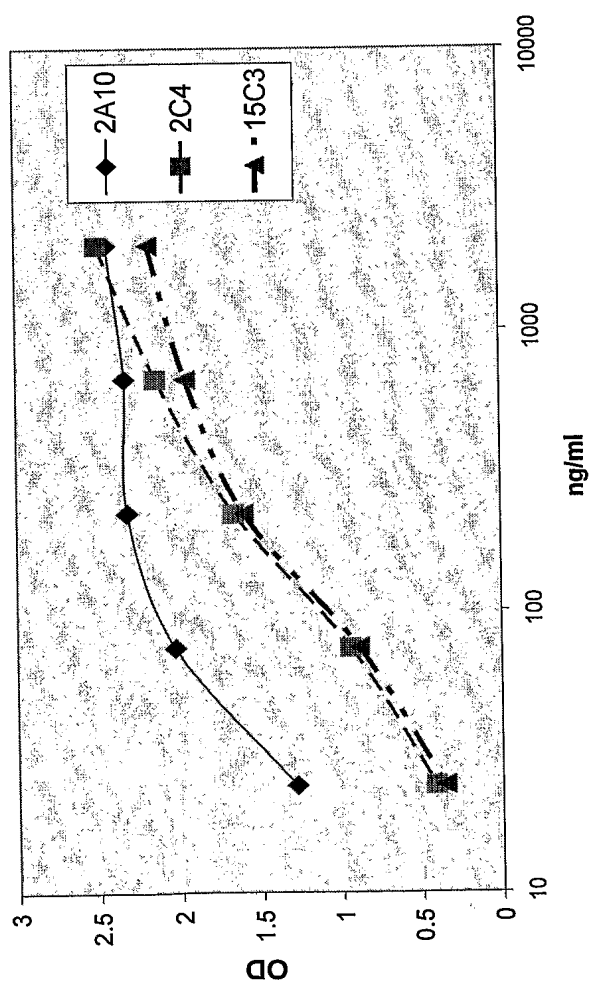
**Figure 6**

Figure 7

**Figure 8**

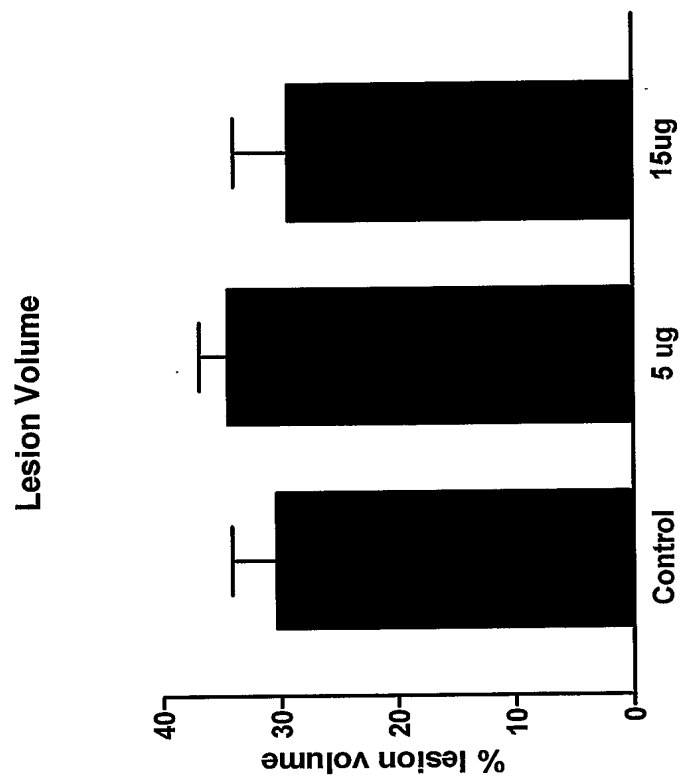
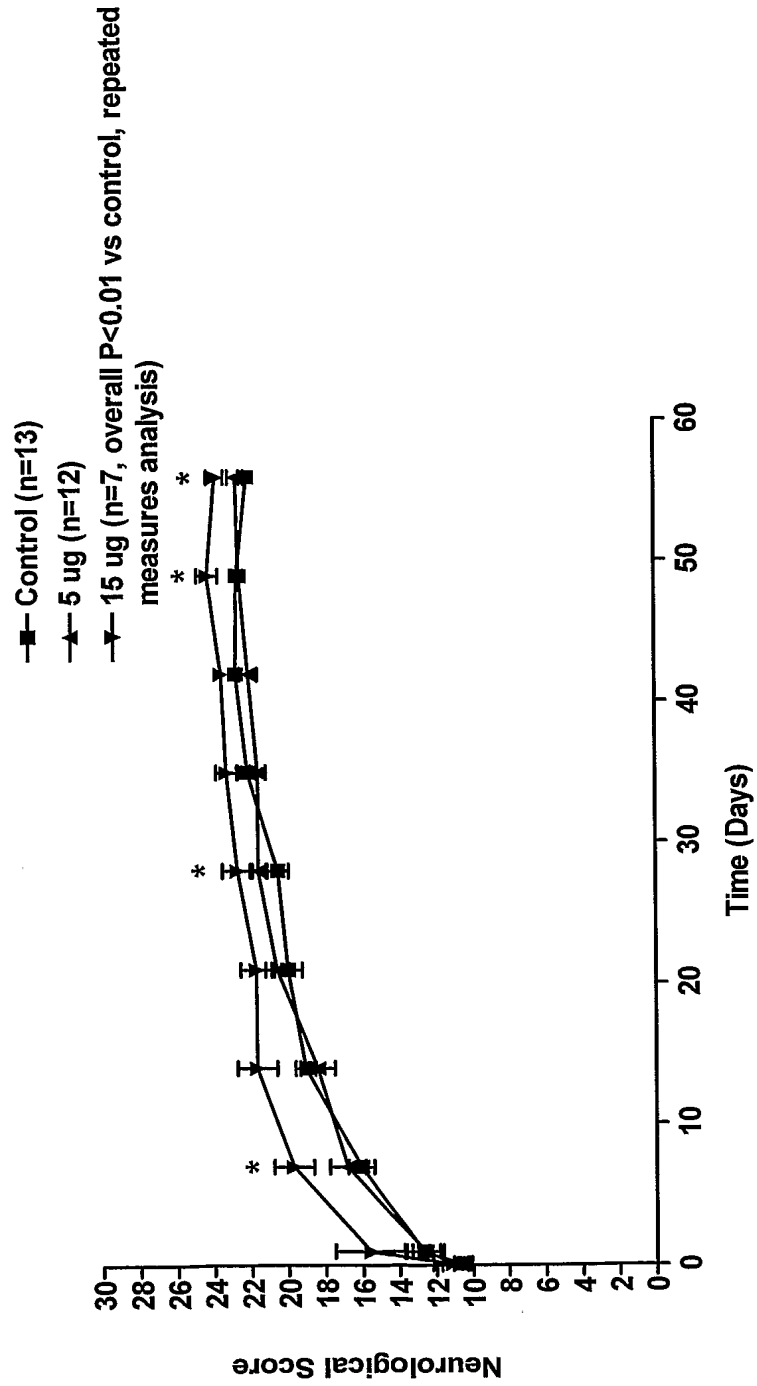
**Figure 9**

Figure 10

* P < 0.05 vs control, data represented as means +/- SEM

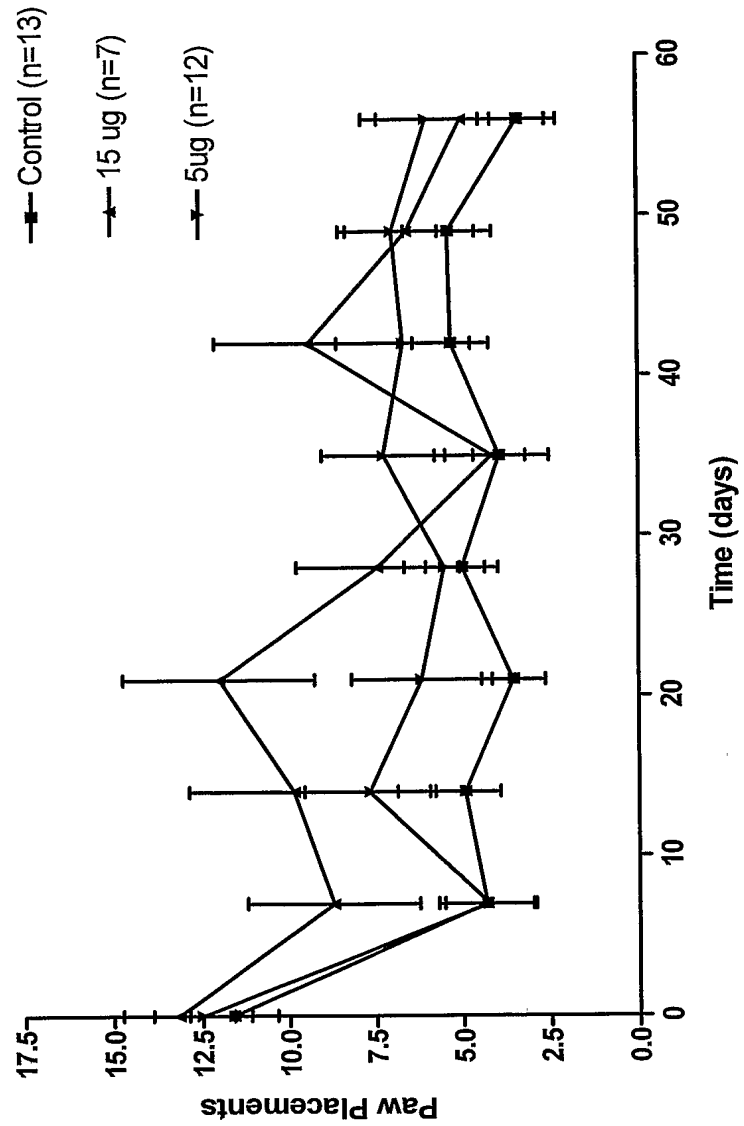
**Figure 11A**

Figure 11B

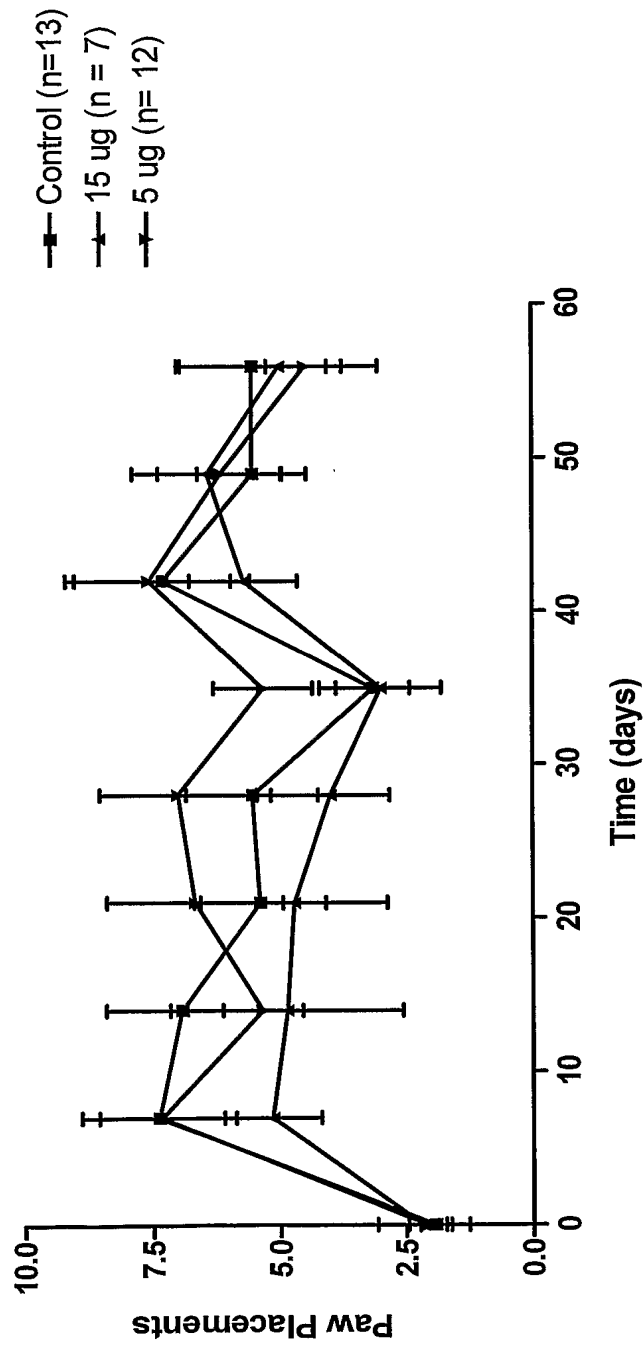
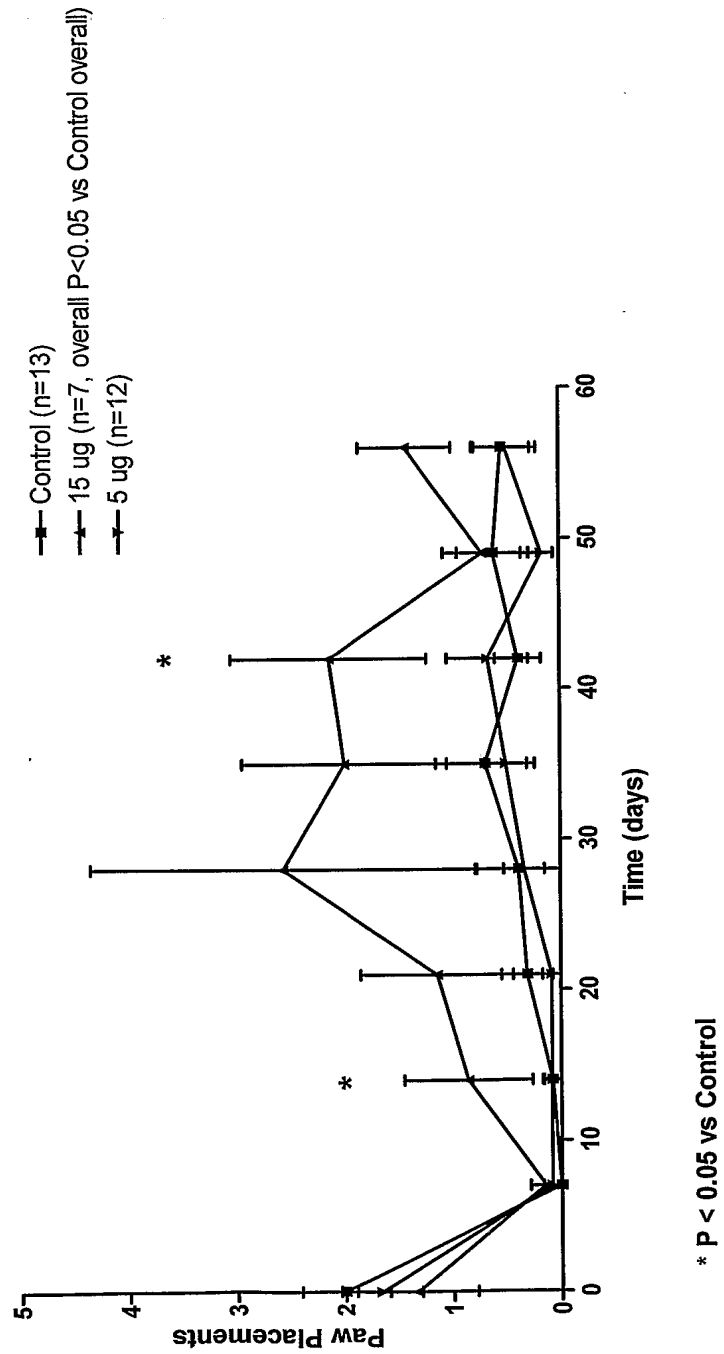


Figure 11C



13/45

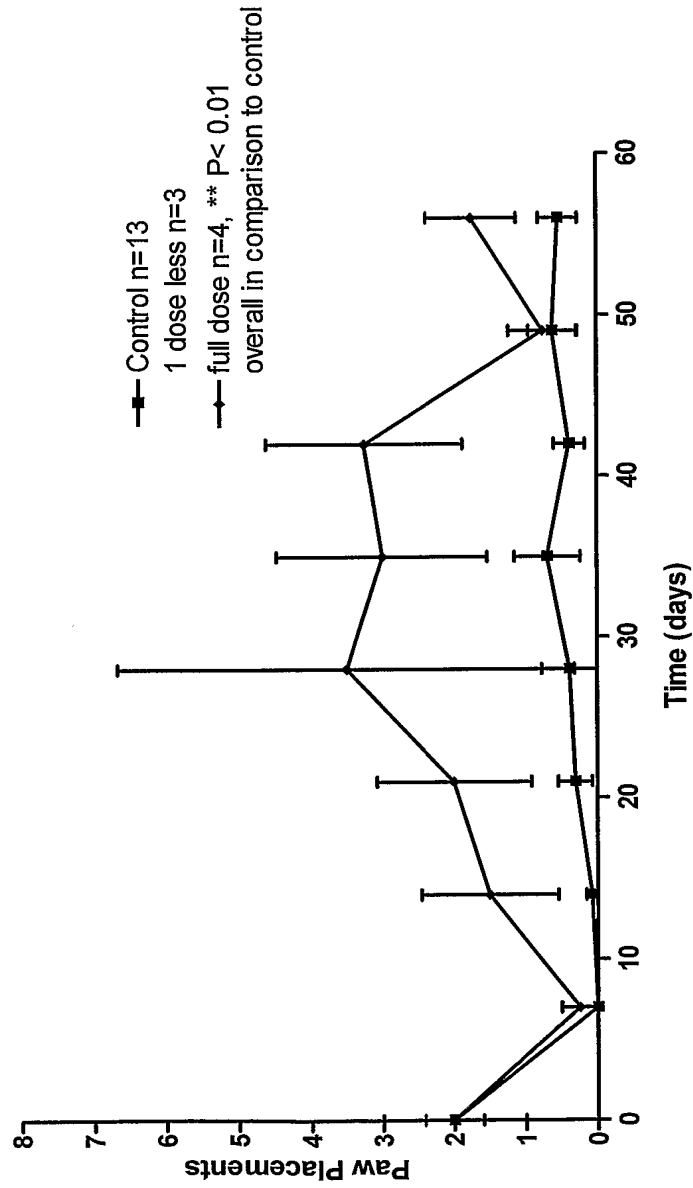


Figure 11D

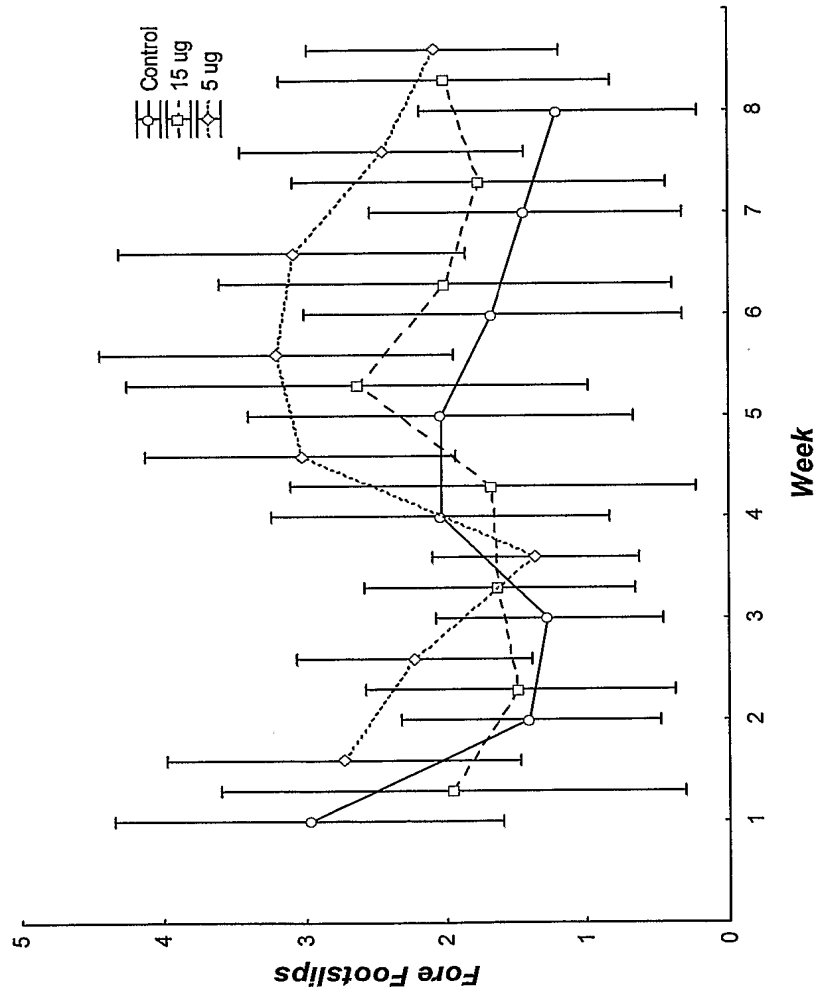


Figure 12

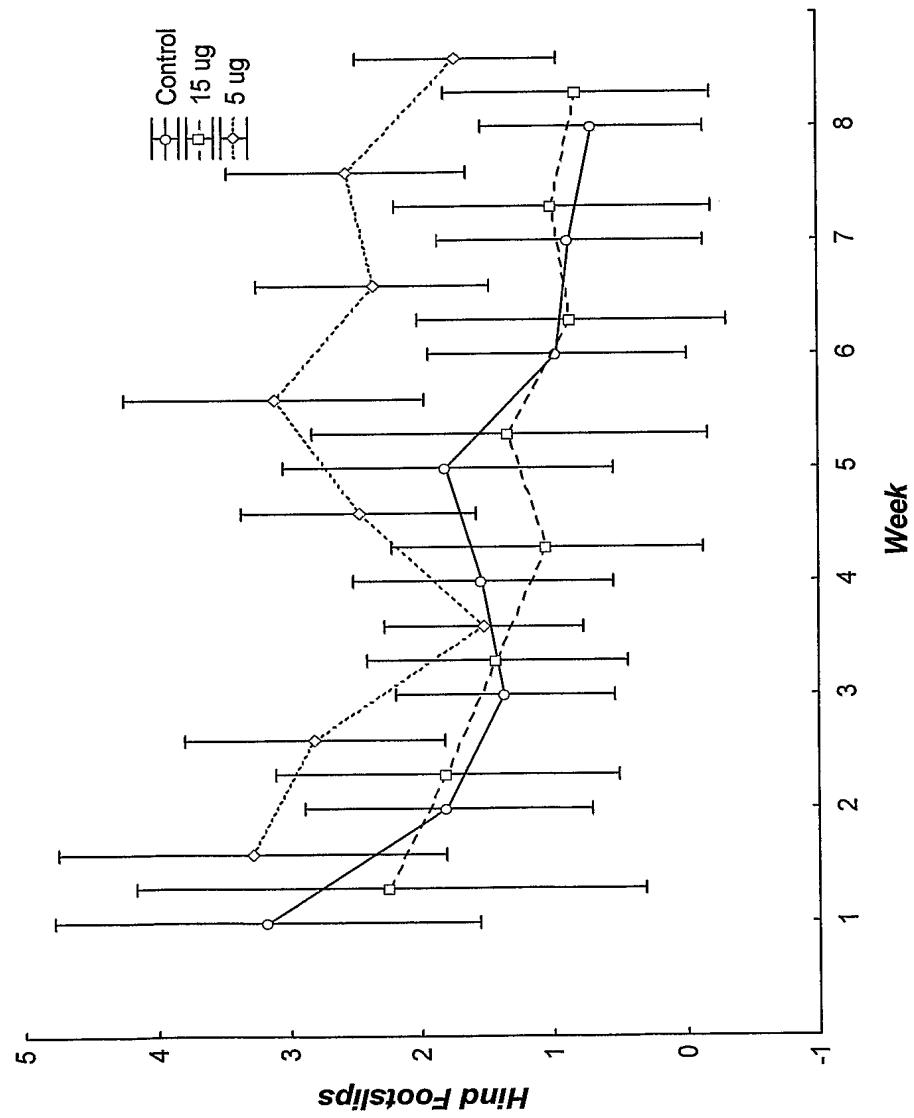
**Figure 13**

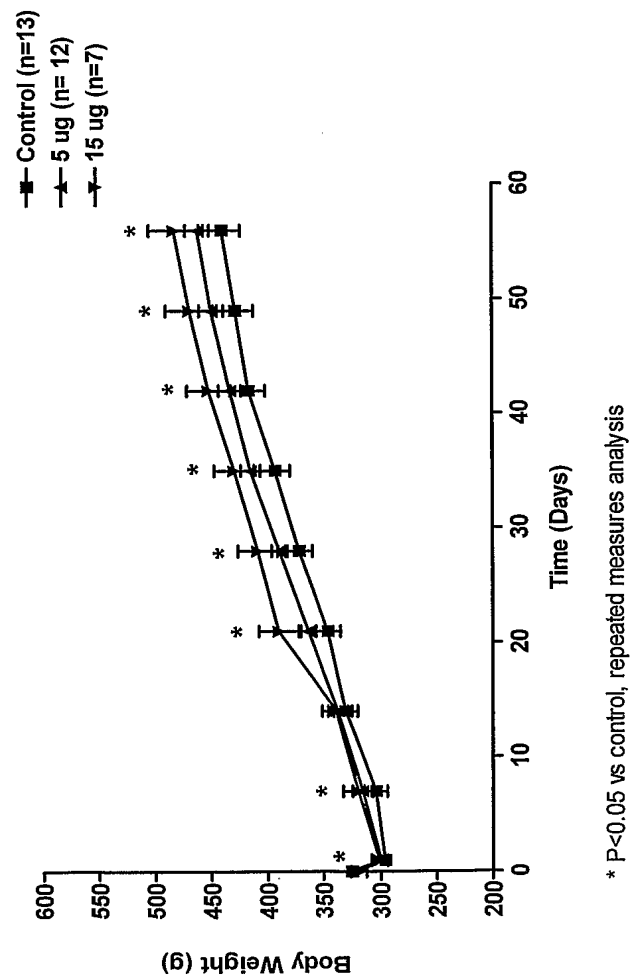
Figure 14A

Figure 14B

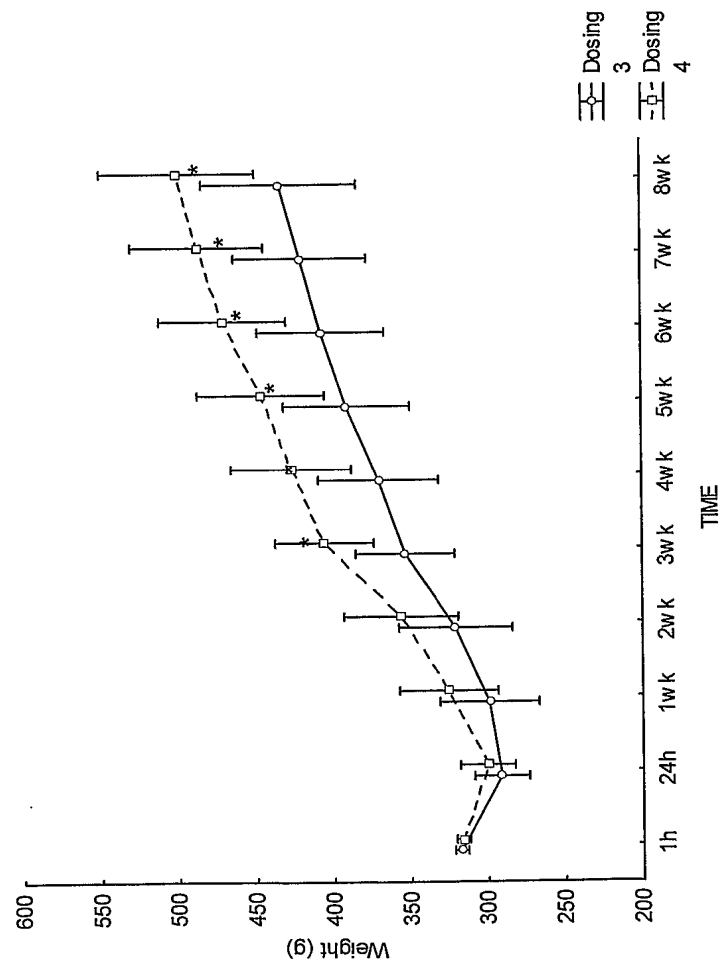
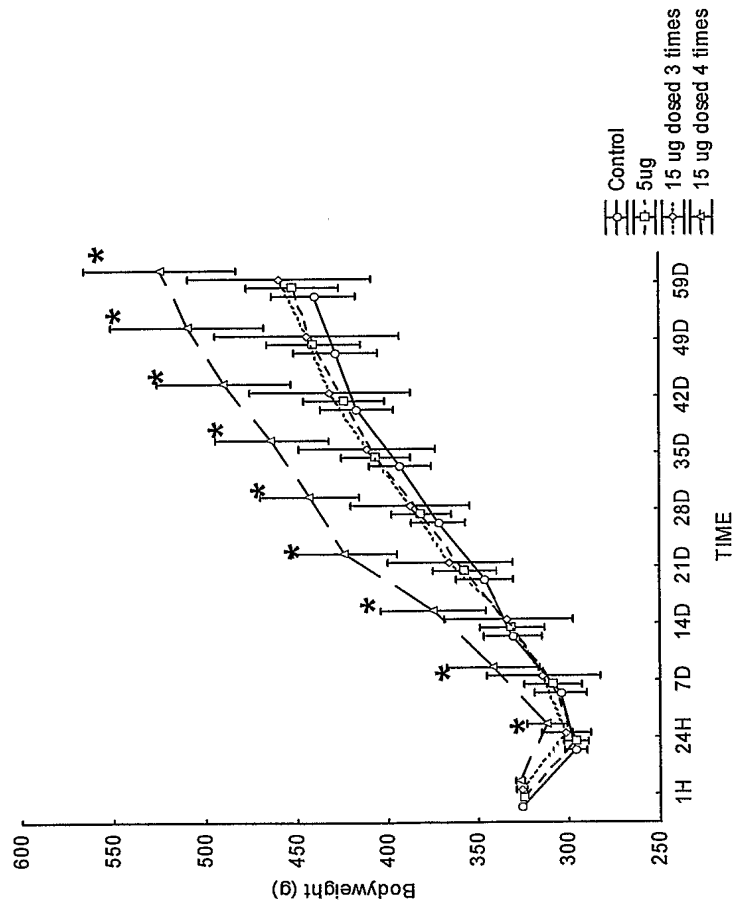


Figure 14C



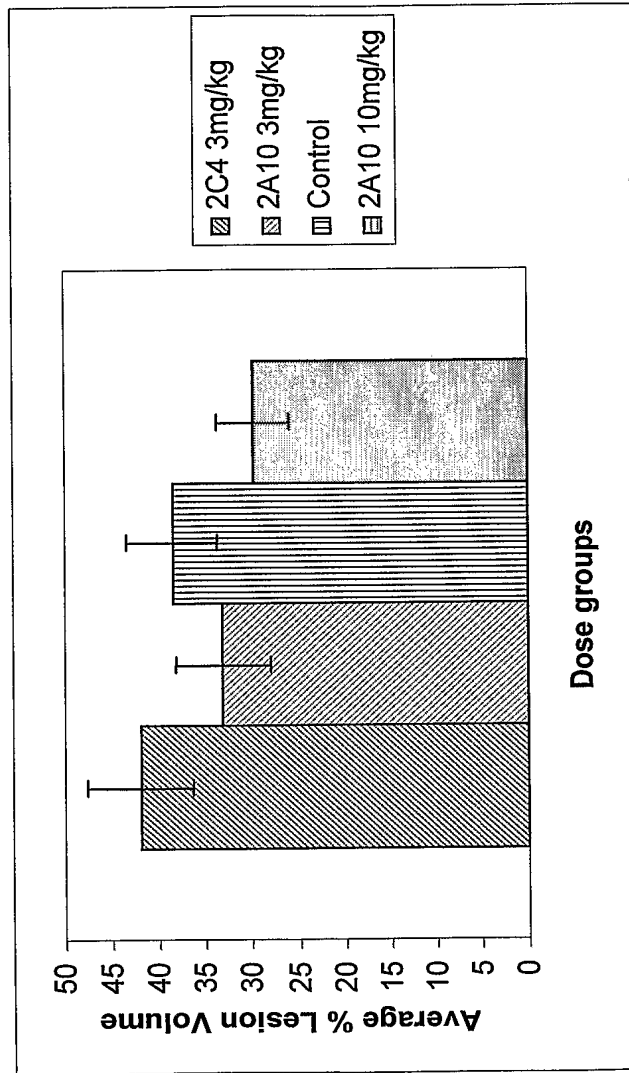
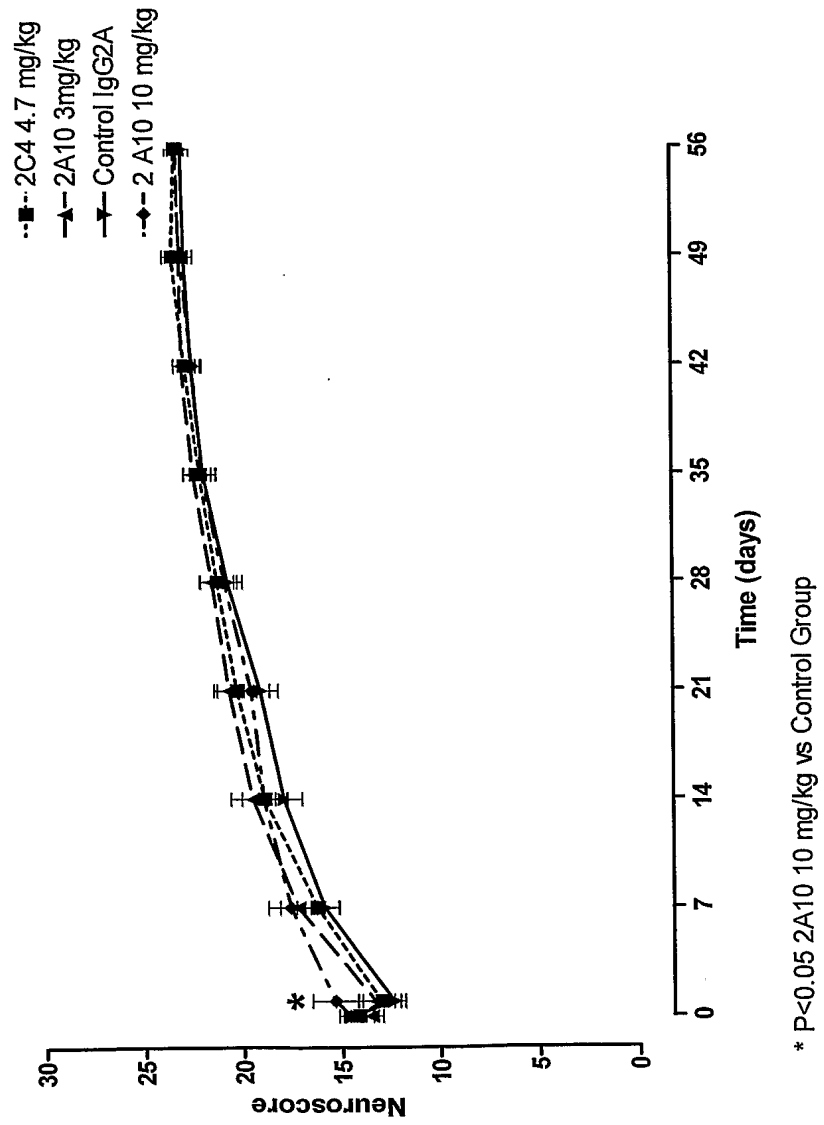
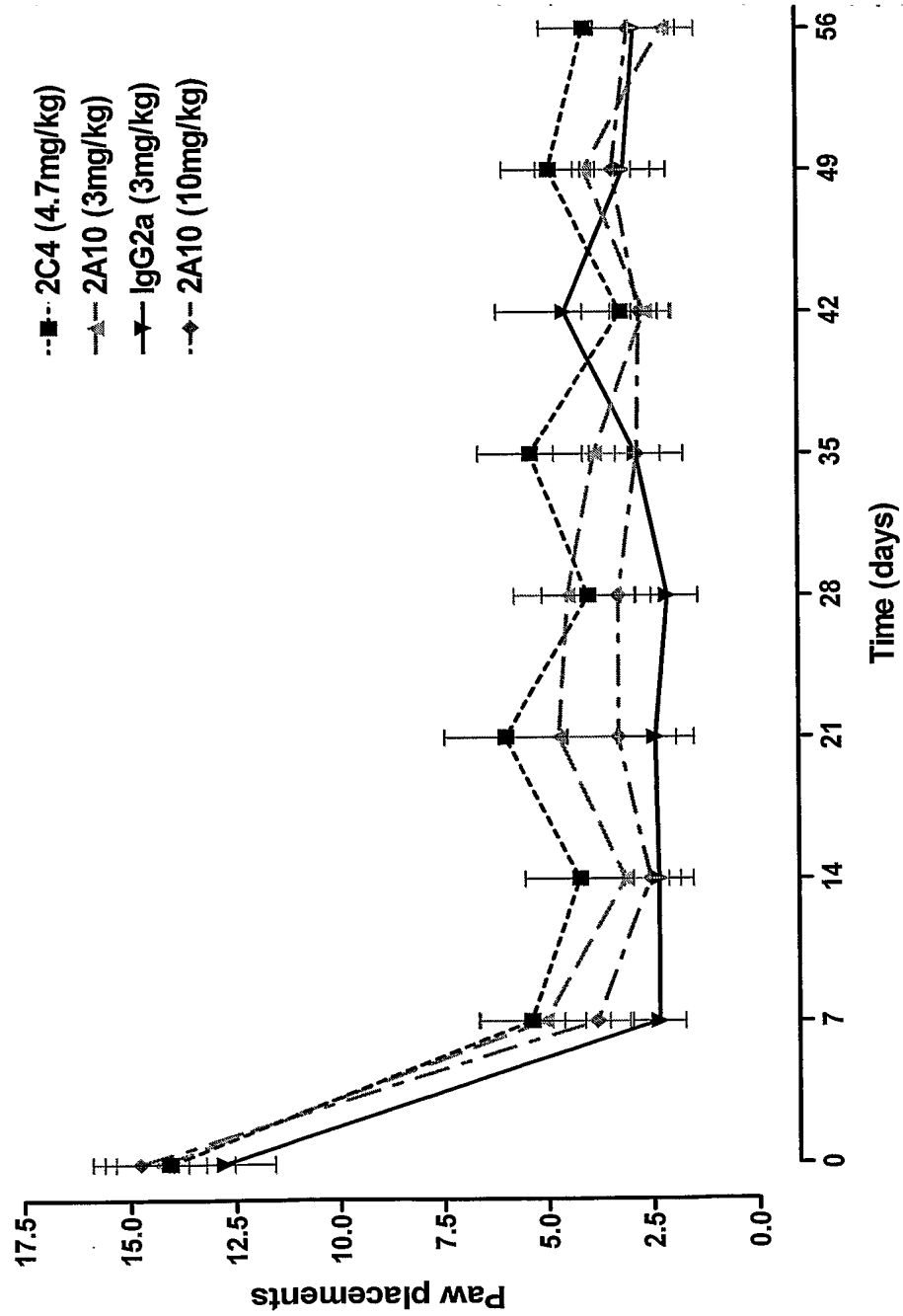


Figure 15

Figure 16



**Figure 17A**

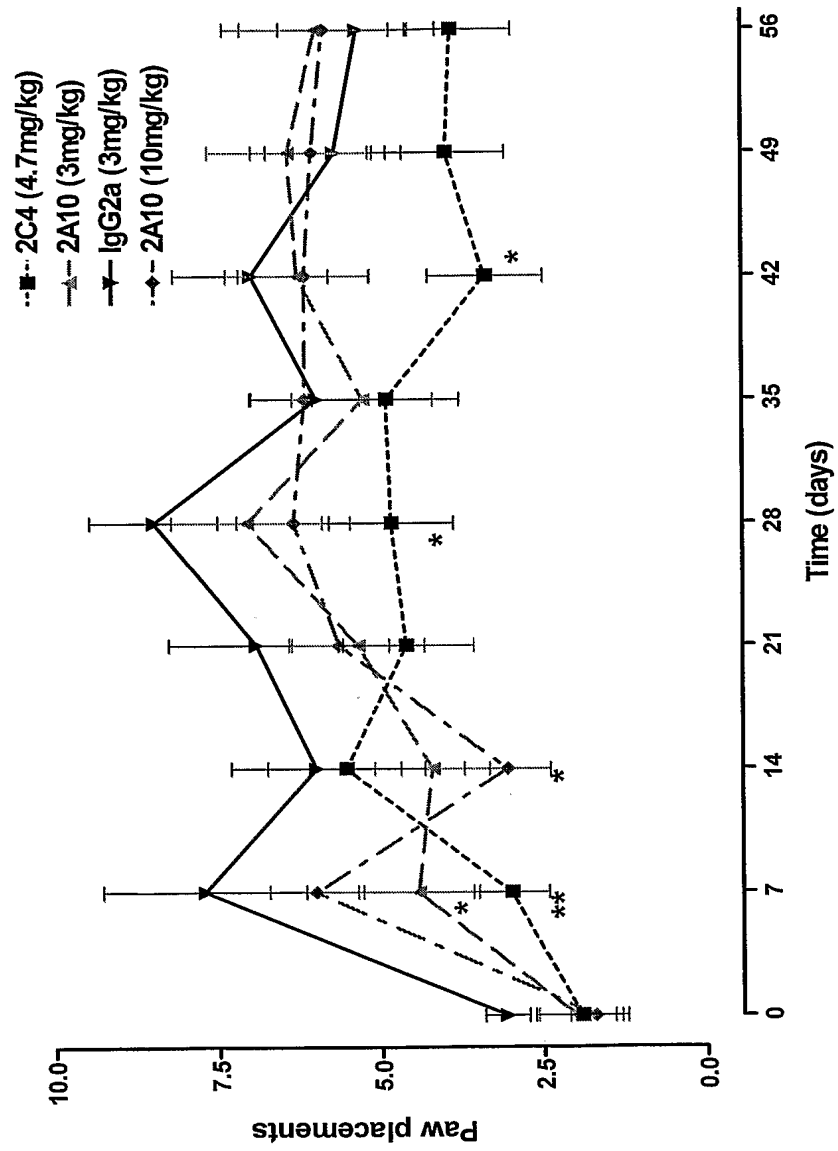


Figure 17B

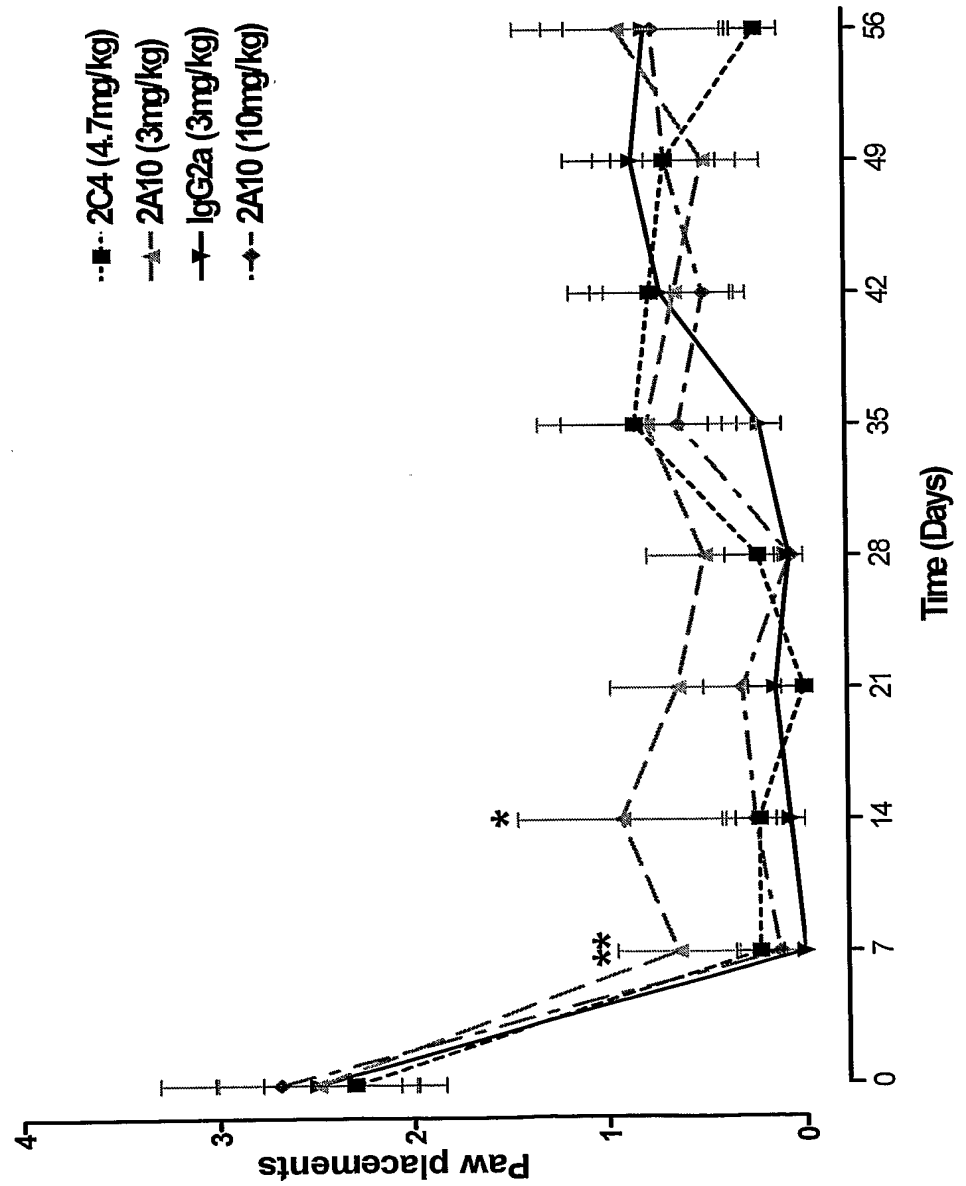


Figure 17C

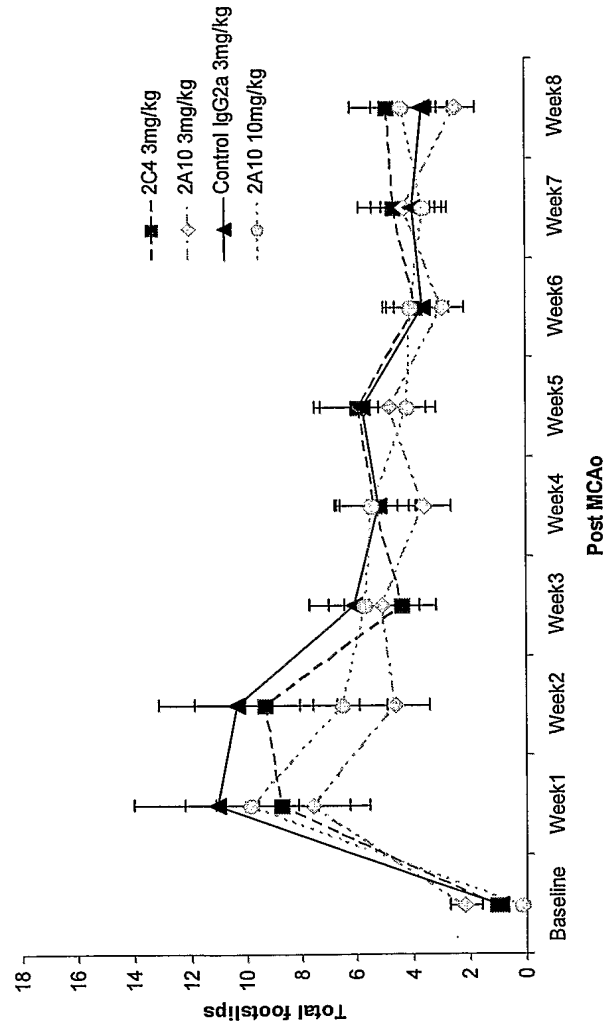
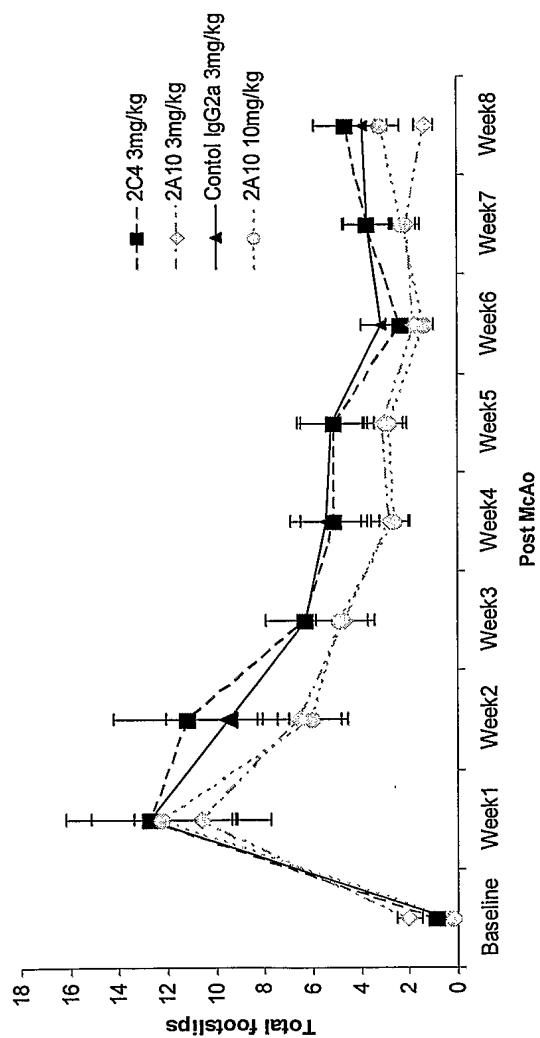


Figure 18

**Figure 19**

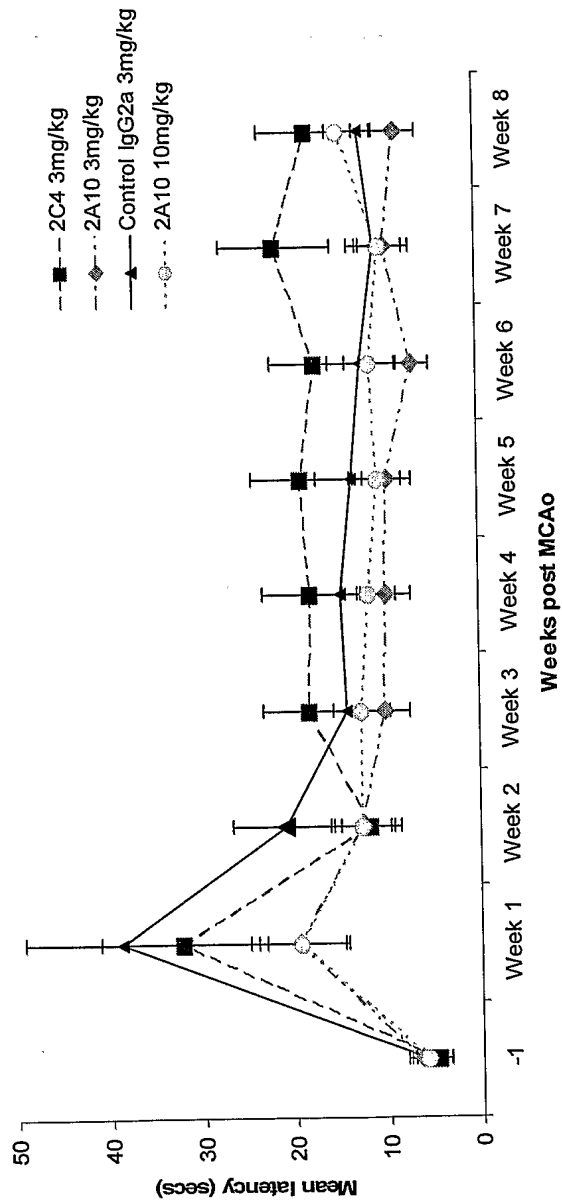
**Figure 20**

Figure 21

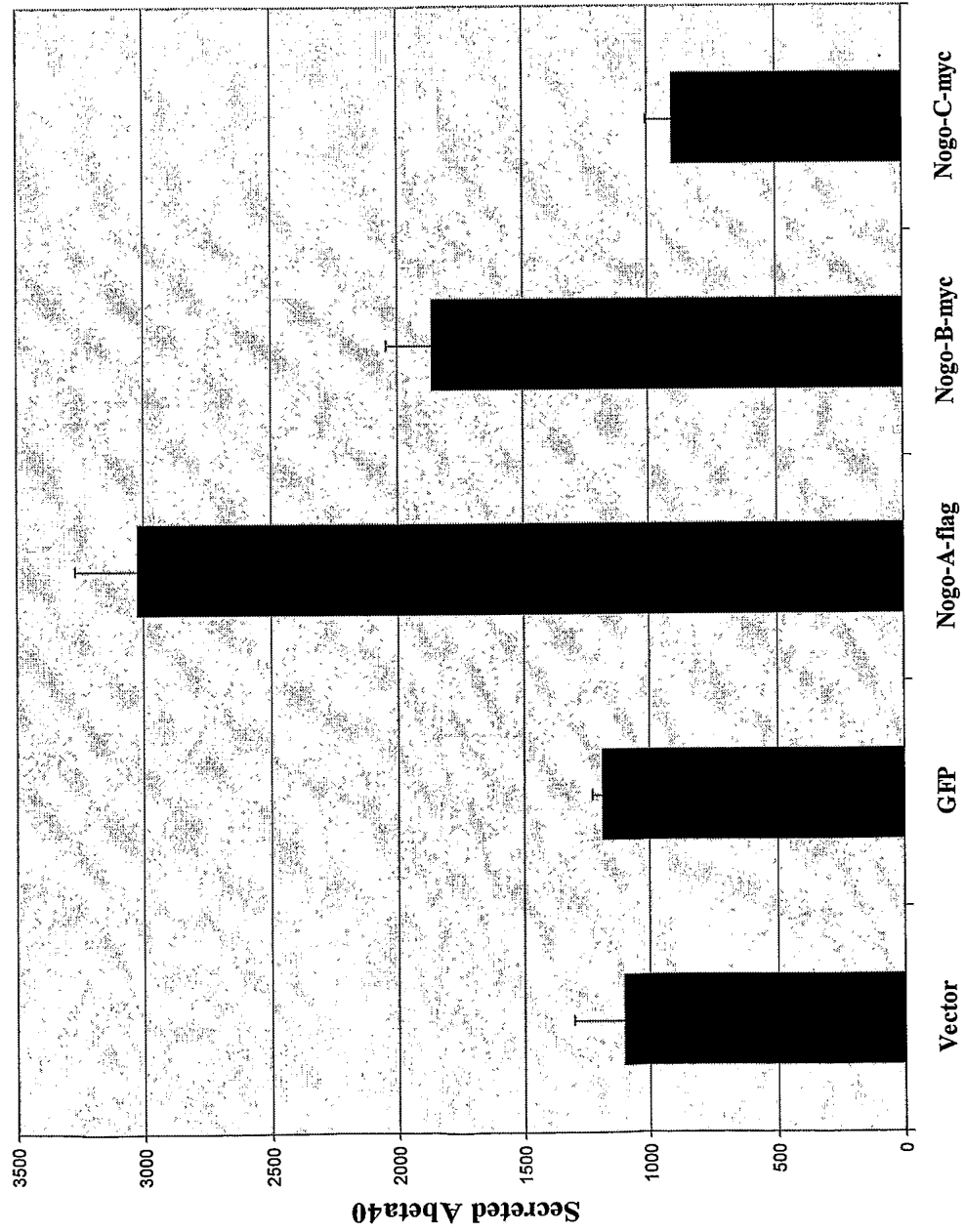


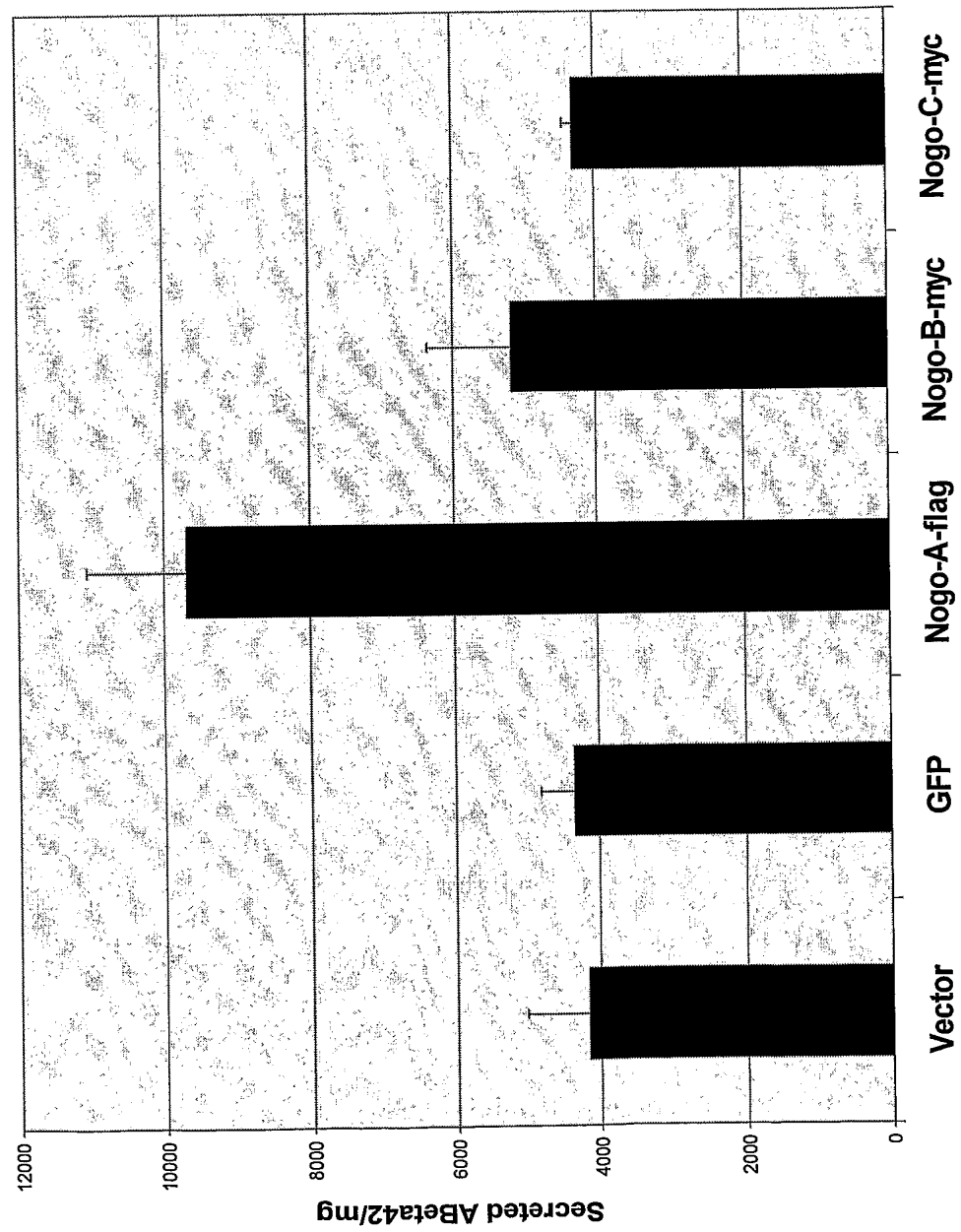
Figure 22

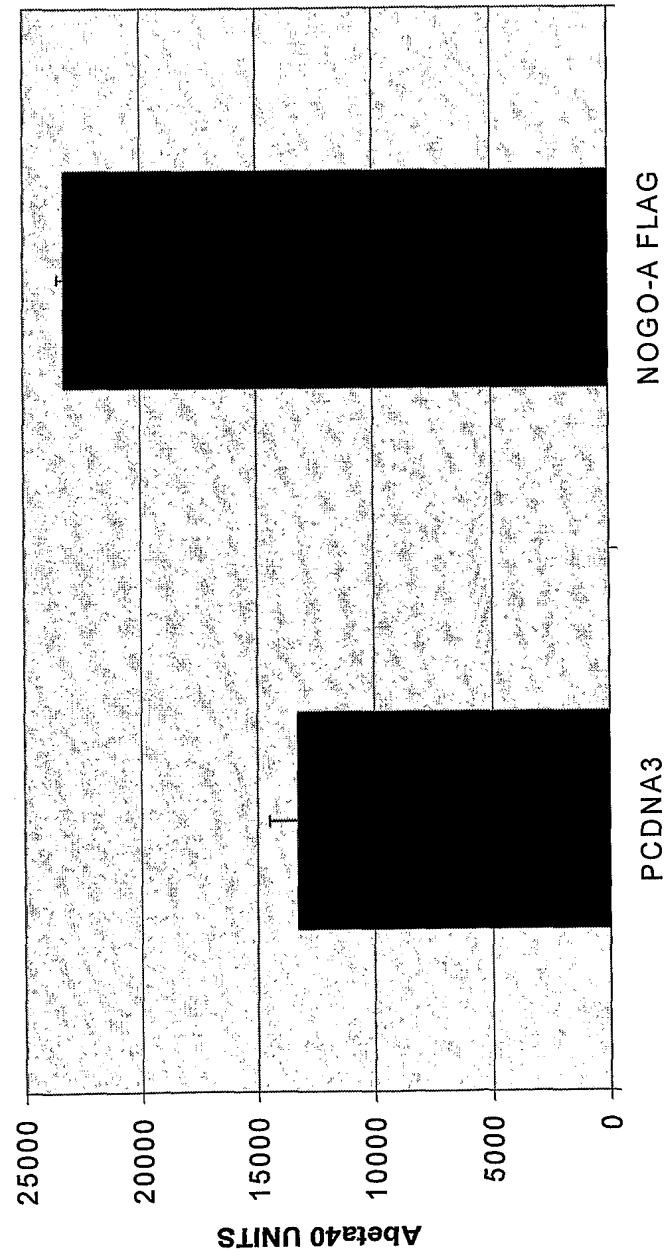
Figure 23

Figure 24

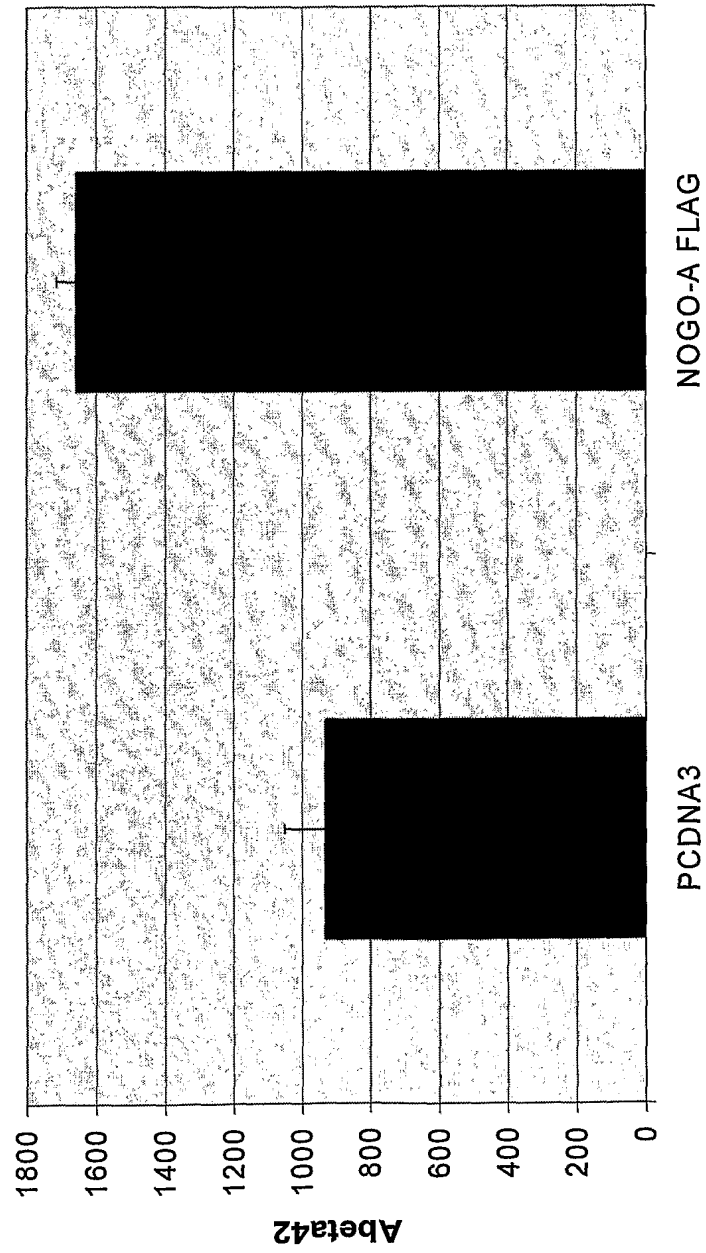


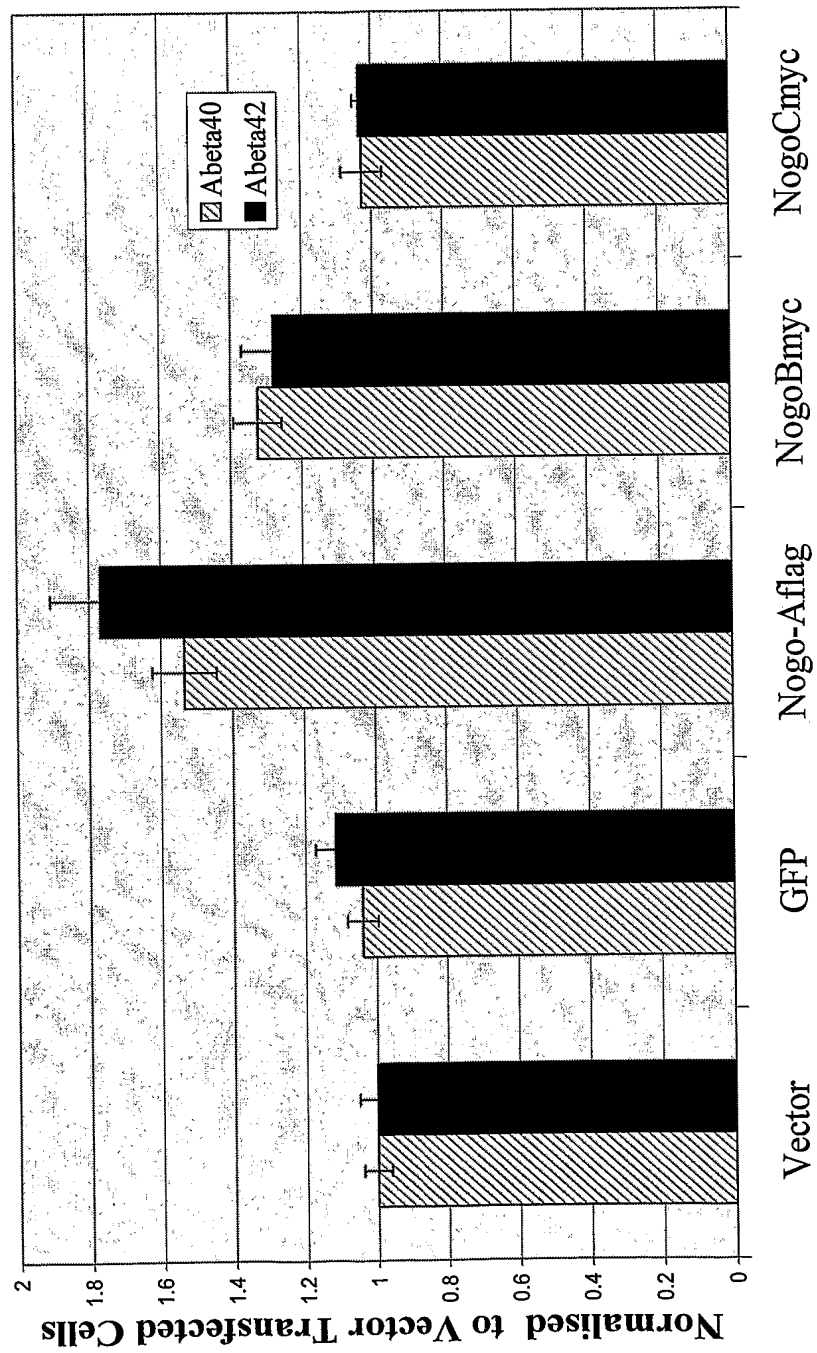
Figure 25

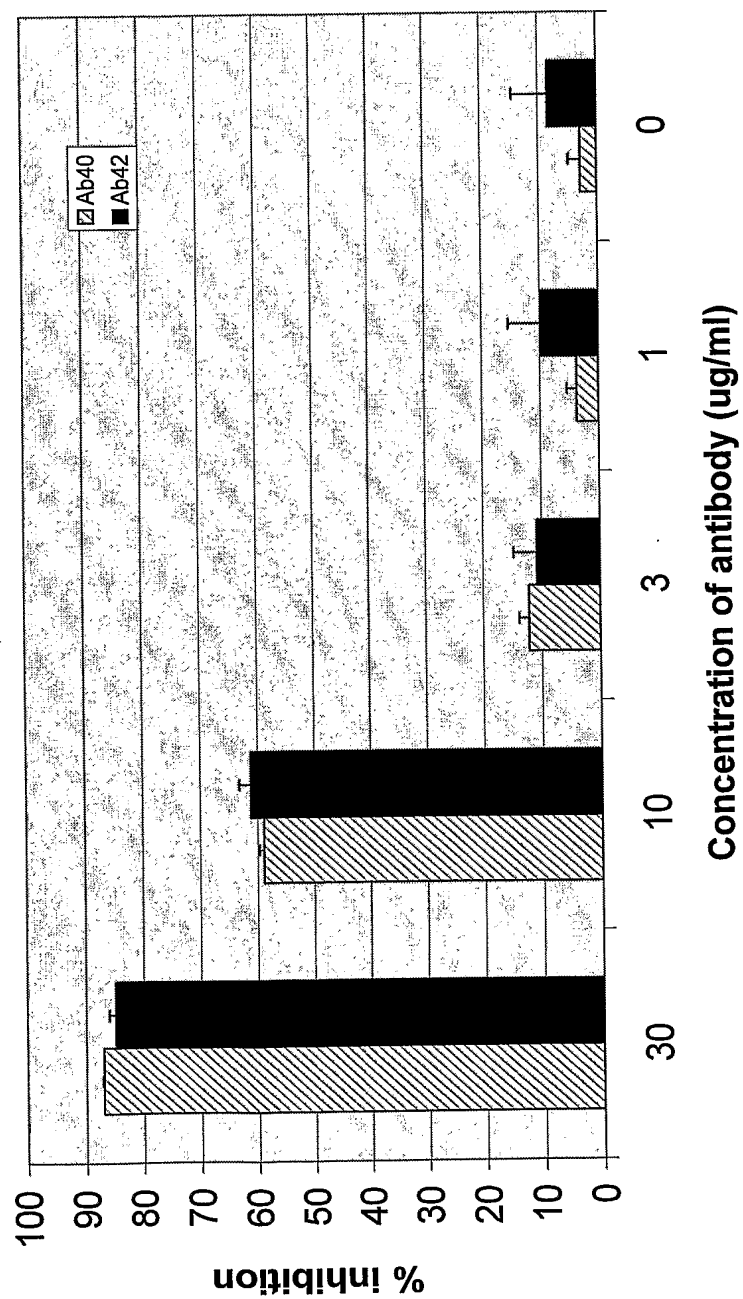
Figure 26

Figure 27

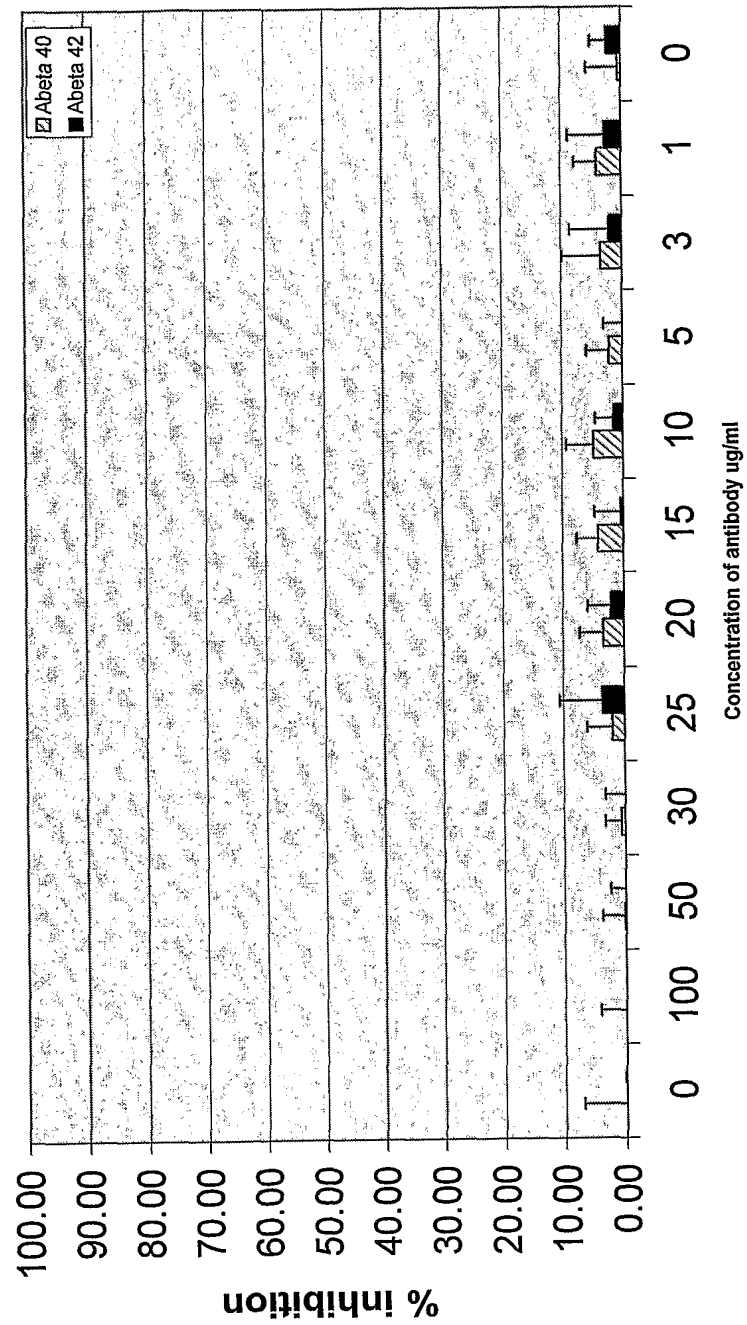


Figure 28

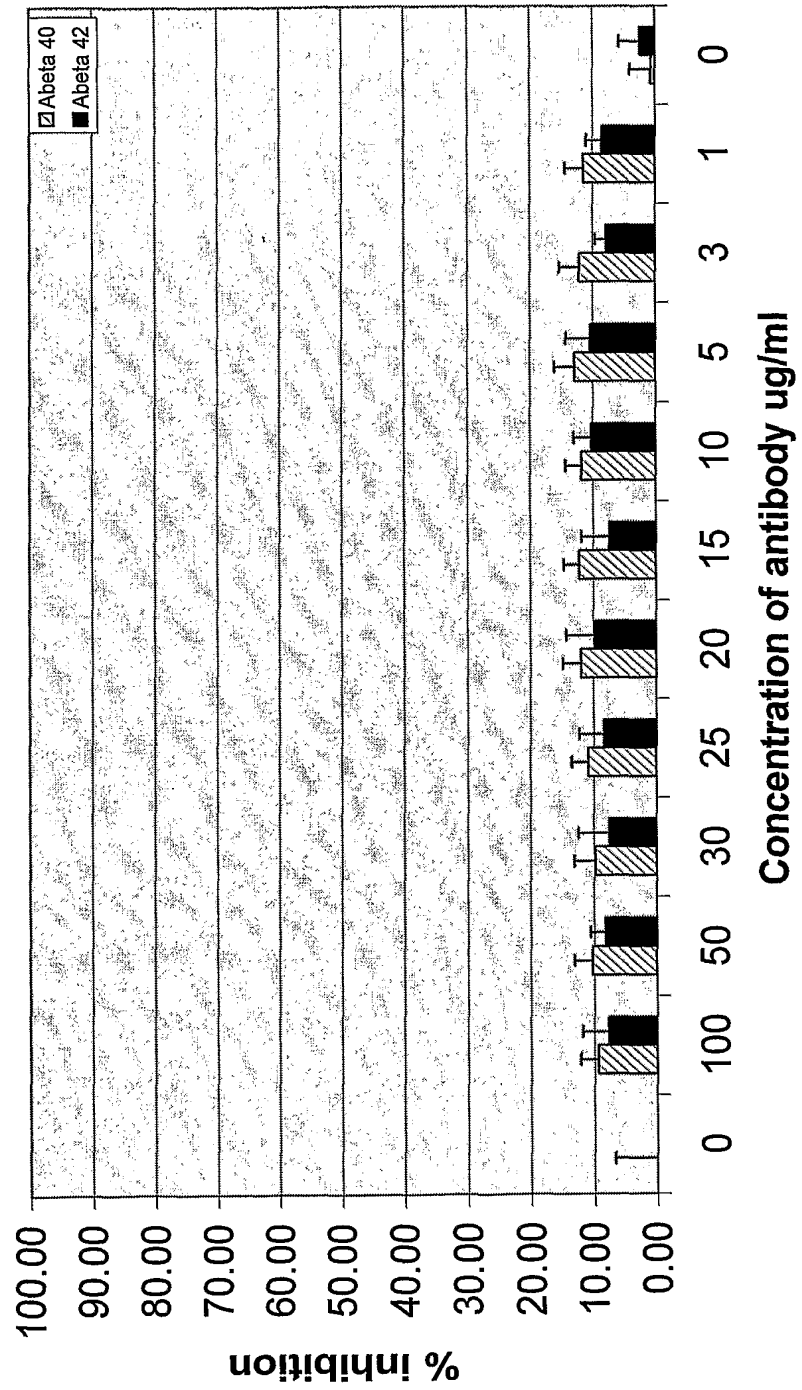


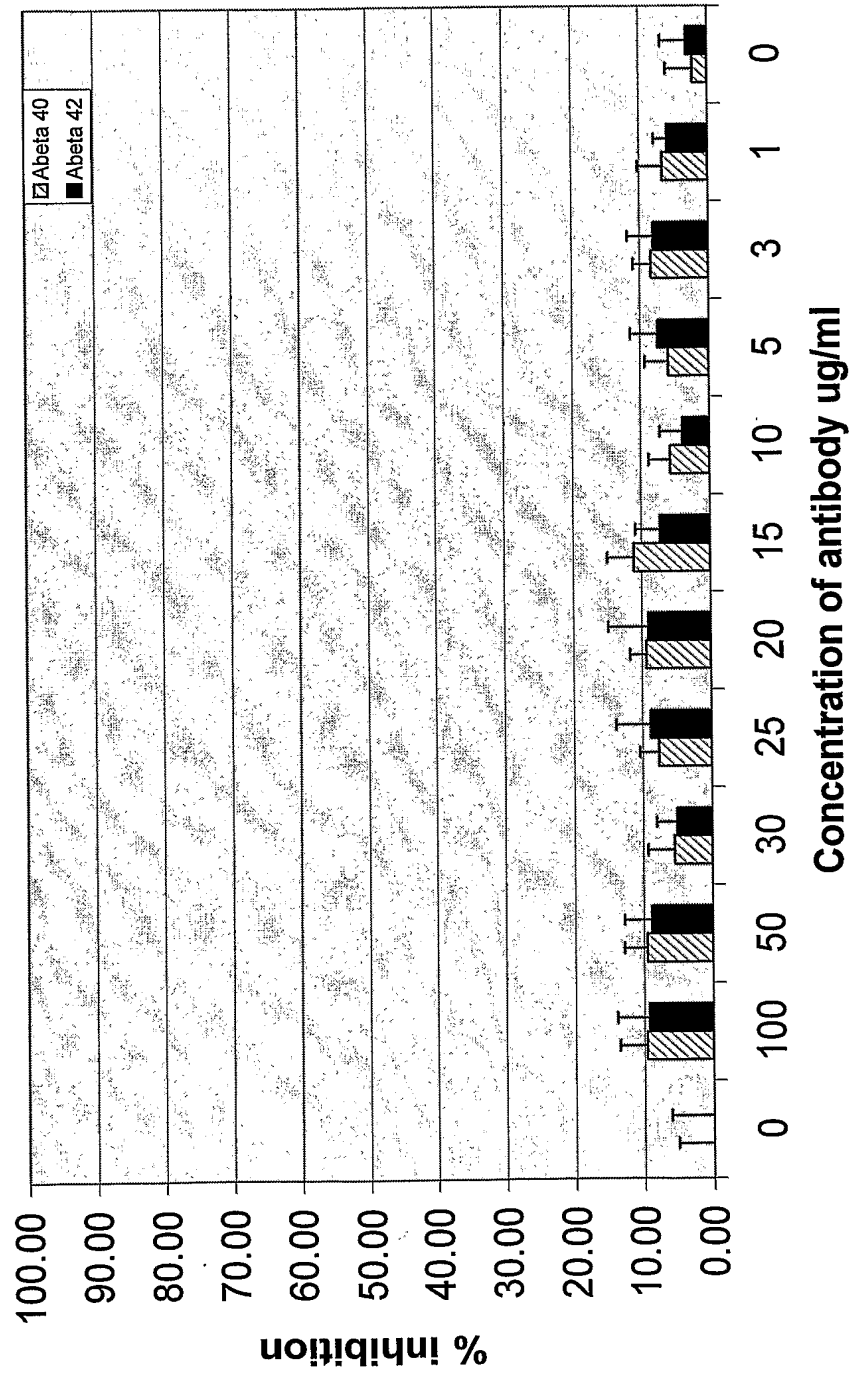
Figure 29

Figure 30

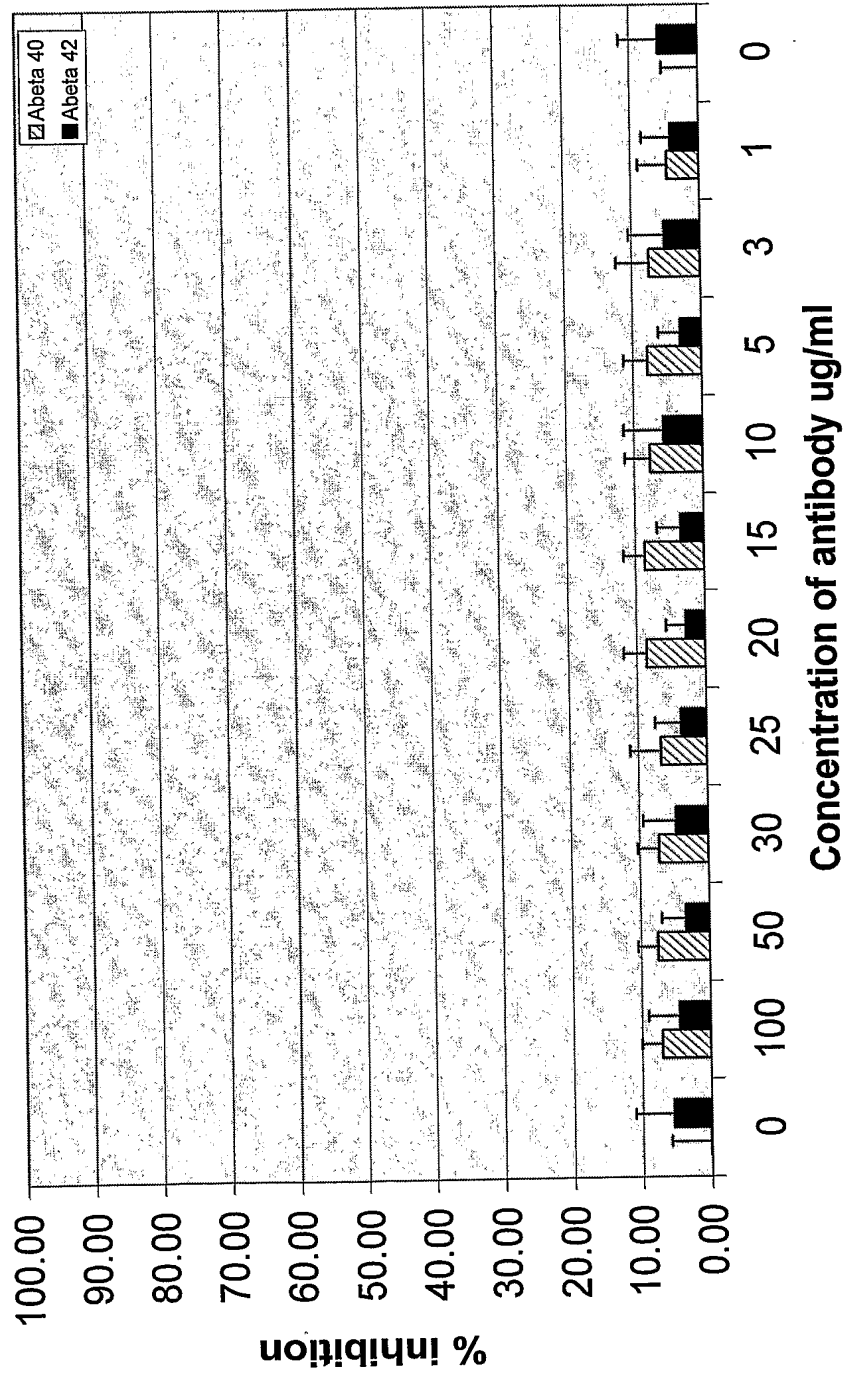


Figure 31

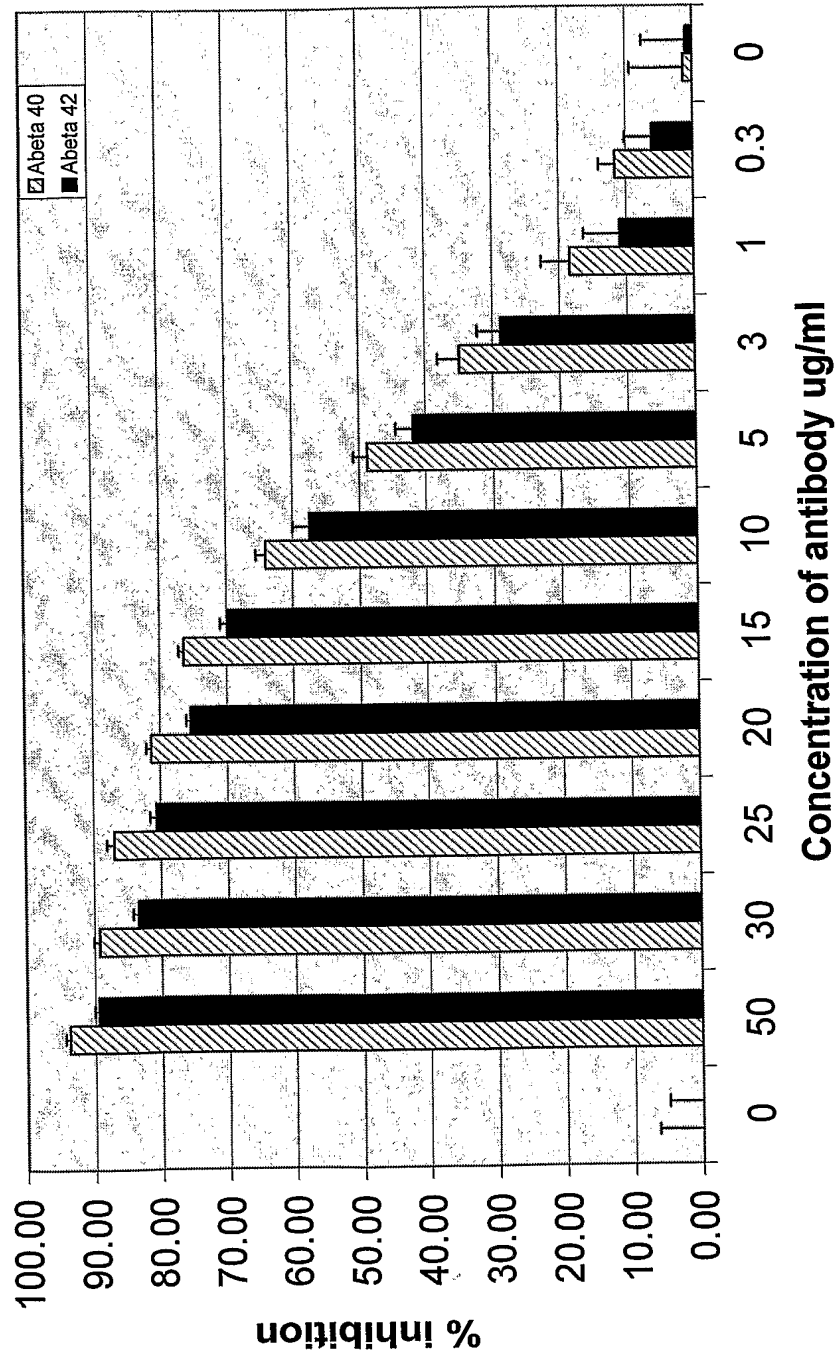


Figure 32

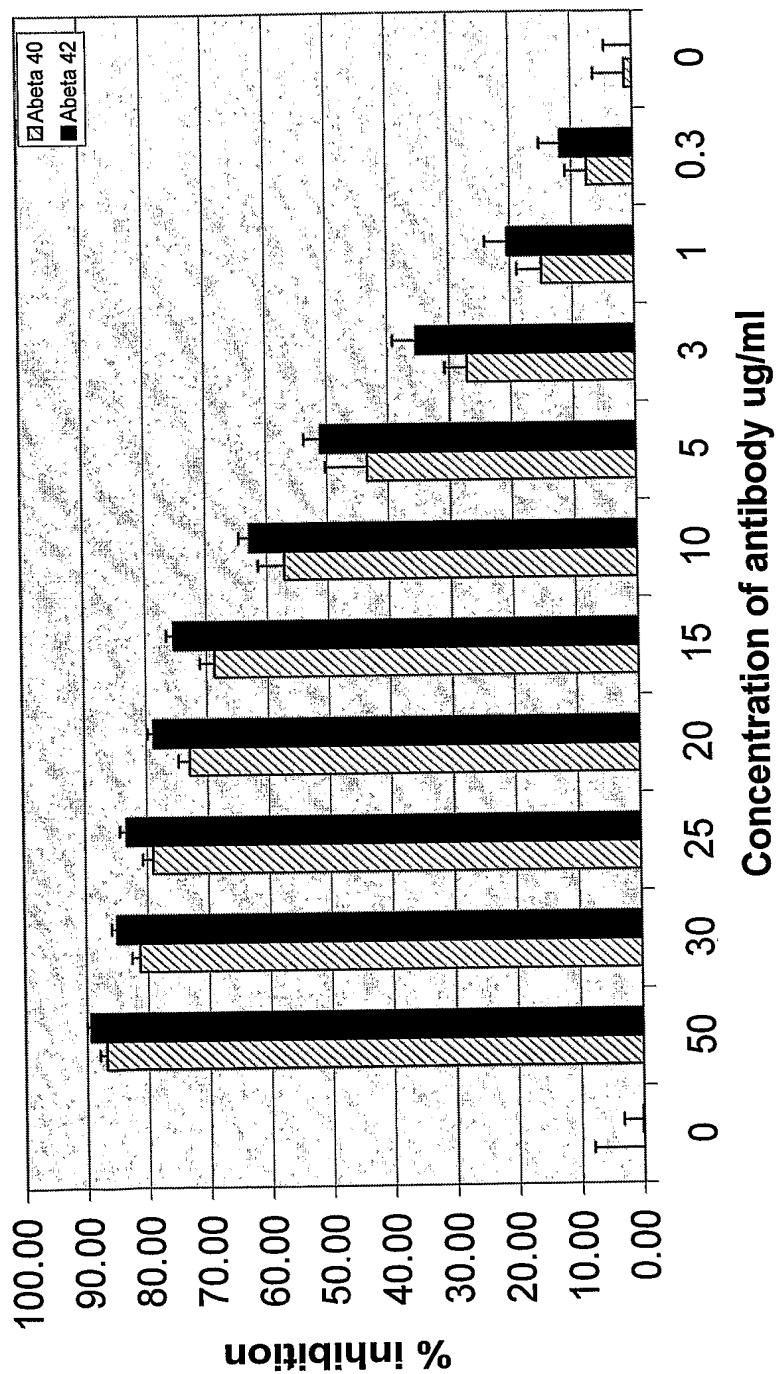


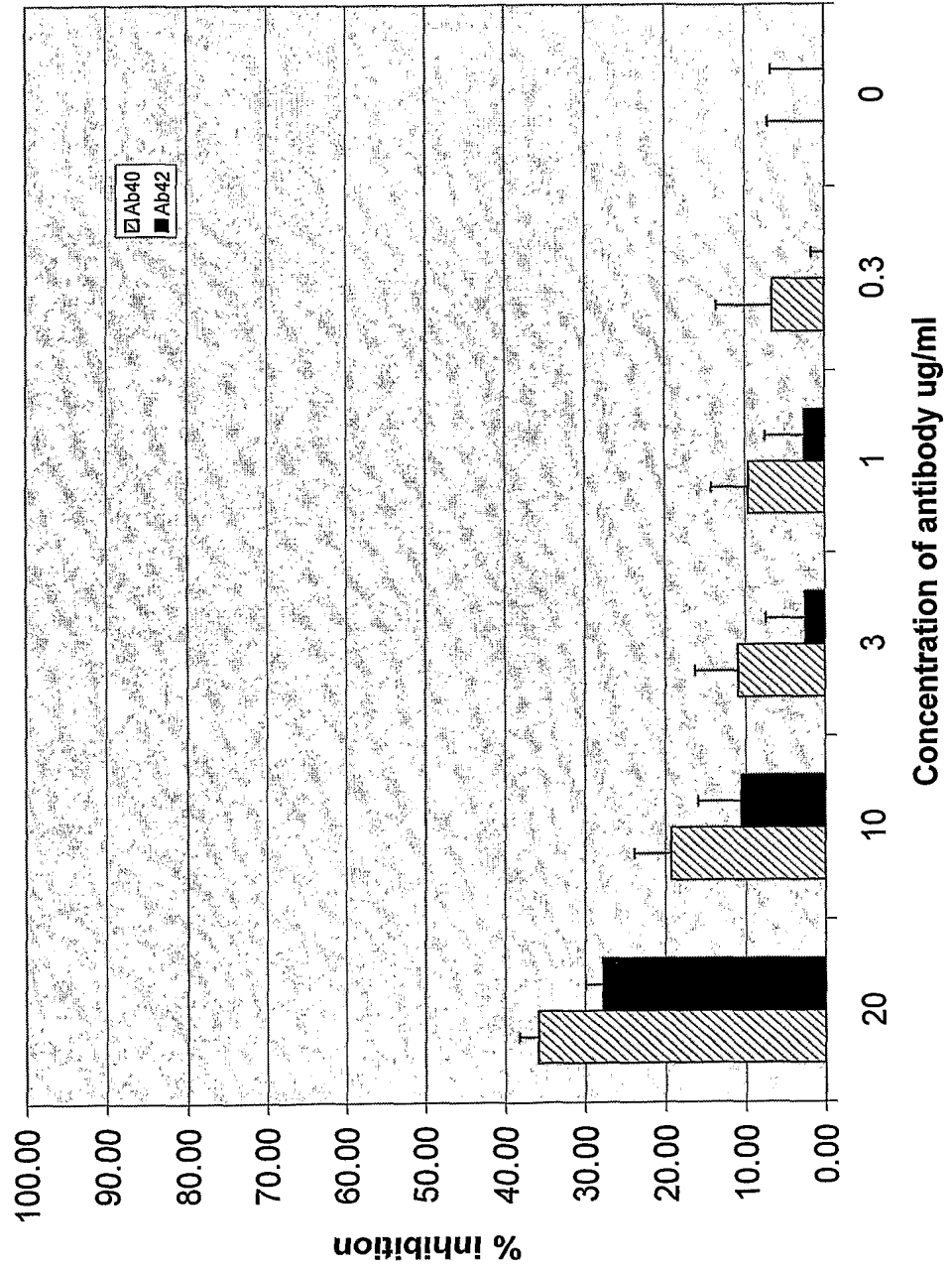
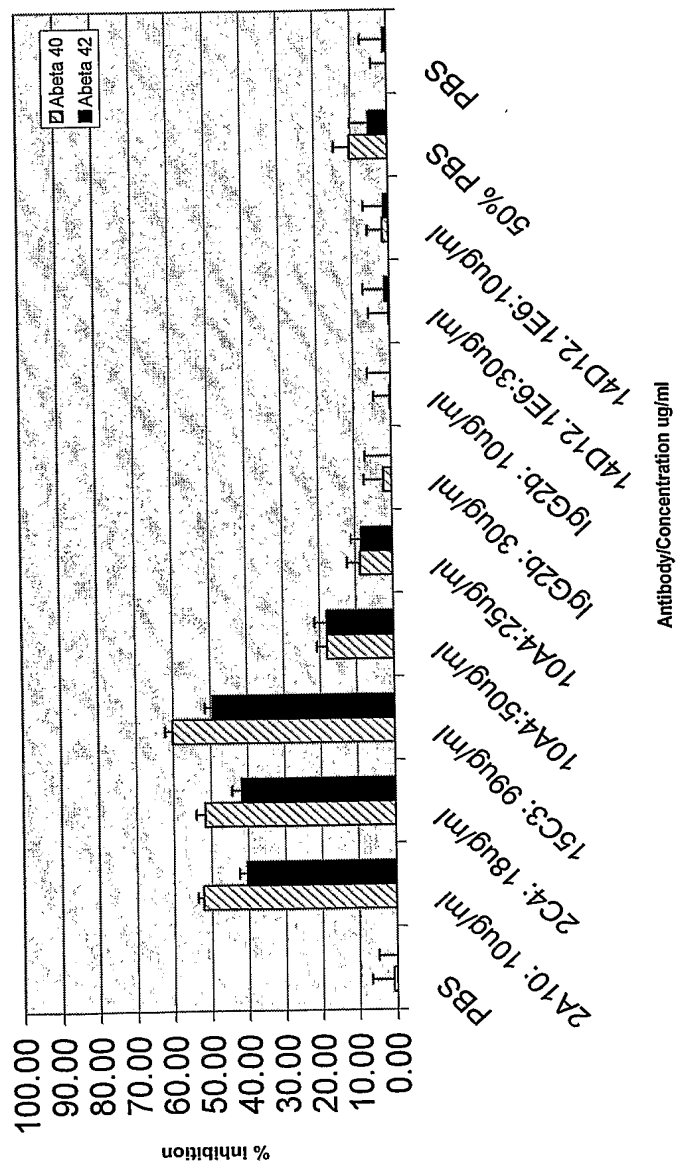
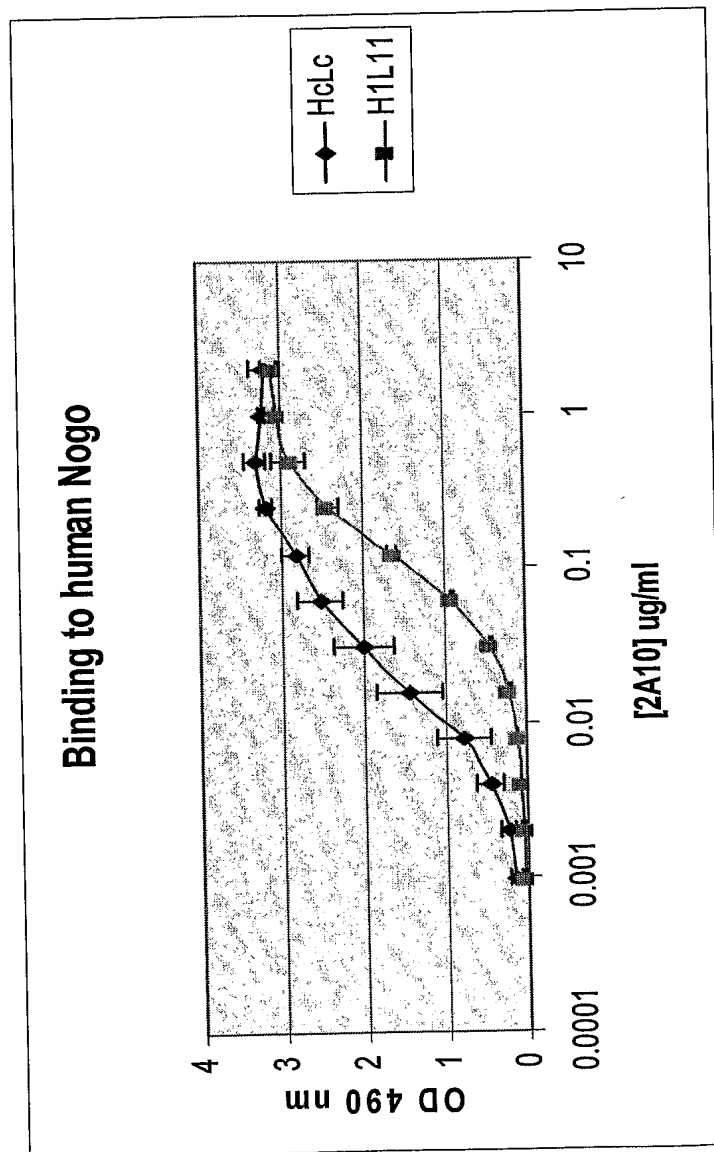
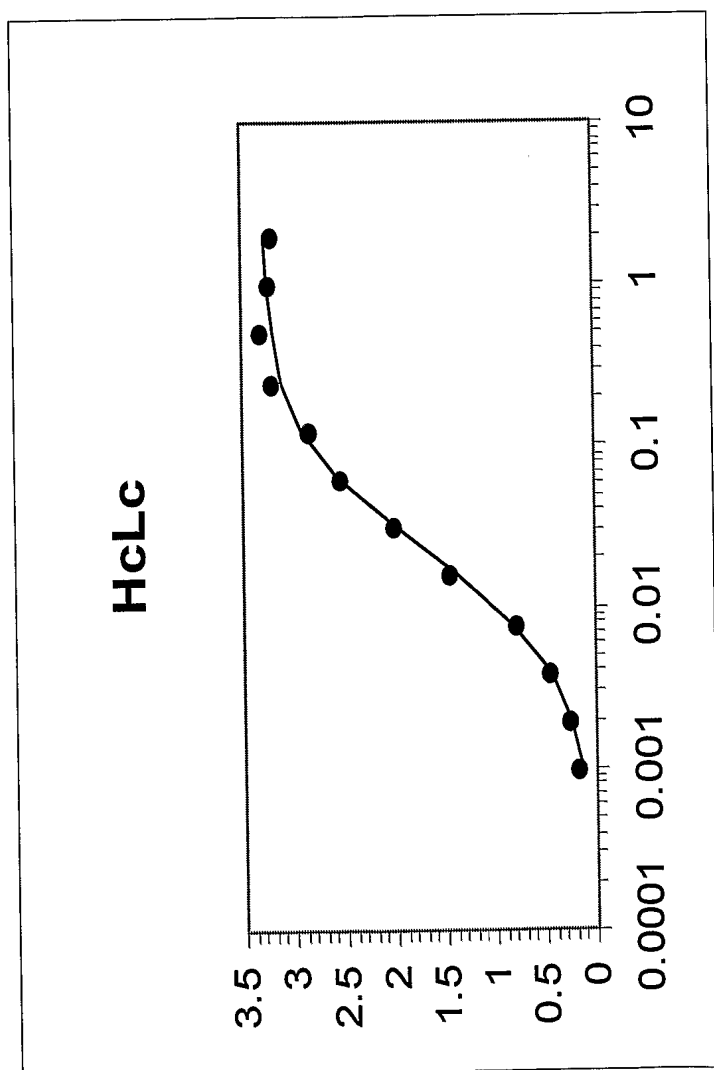
Figure 33

Figure 34

**Figure 35A**

**Figure 35B**

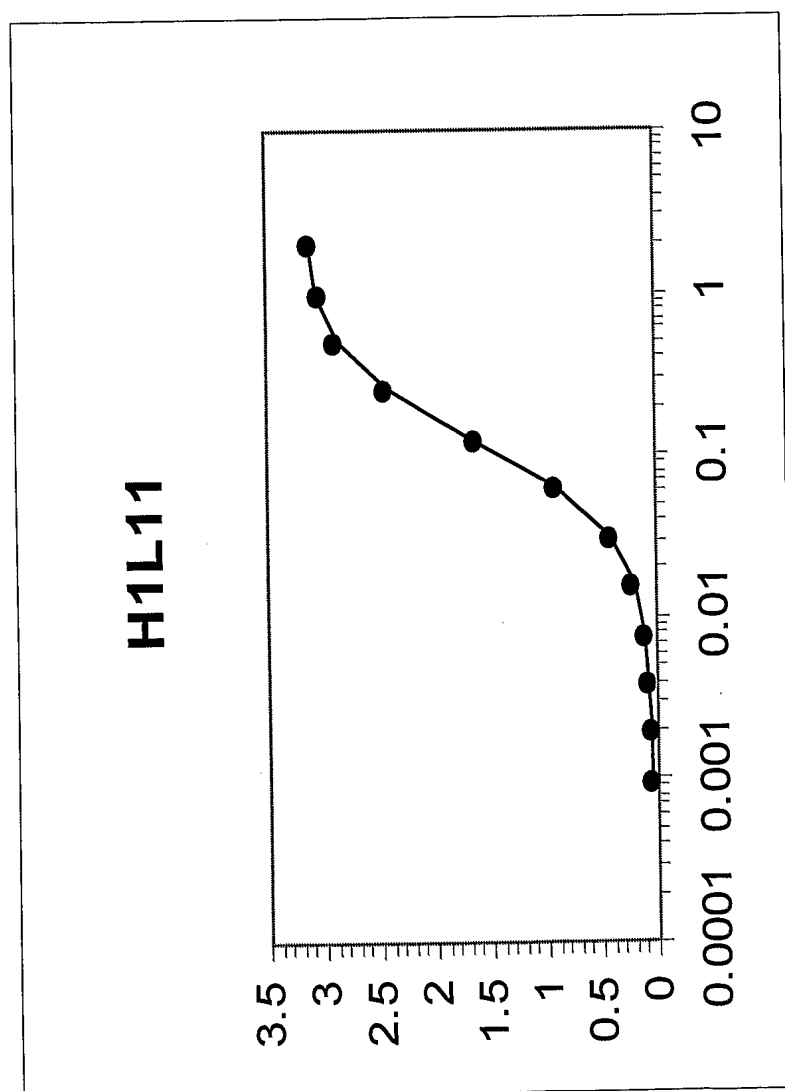


Figure 35C

Figure 36

NogoAmyc (μg):

